Sequence and Relationships of Sugarcane Mosaic and Sorghum Mosaic Virus Strains and Development of RT-PCR–Based RFLPs for Strain Discrimination

Z. N. Yang and T. E. Mirkov

Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, Texas A&M University System, 2415 E. Highway 83, Weslaco 78596.

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ABSTRACT

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Sugarcane mosaic is the most widespread virus disease affecting sugarcane production. We have established a collection of seven prominent sugarcane mosaic potyvirus (SCMV) strains currently causing disease in sugarcane throughout the world and originally found in sugarcane in the United States. This collection includes SCMV strains A, B, D, and E, and the sorghum mosaic virus (SrMV) strains SCH, SCI, and SCM. These viruses were propagated on *Sorghum bicolor* cv. Rio and purified. Cloned cDNAs representing 2.0 kb of the 3' termini, obtained after a reverse-transcriptase–polymerase chain reaction (RT-PCR) on purified virions

using an oligo(dT) primer and degenerate primers with sequences located in the N1b gene, have been sequenced for each of these strains. A comparative analysis of the deduced amino acid sequences in the N1b and coat protein genes and of the nucleotide sequences in the 3'-untranslated region, among these seven viruses and among six other members of the SCMV subgroup, confirms that there are at least four, but suggests that there are five, distinct viruses in the SCMV complex. Based on these seven new sequences and on the available sequence data for six other members of the SCMV subgroup, we have developed group-specific primers for use in RT-PCR–based restriction fragment length polymorphism analysis for rapid discrimination between strains of SCMV and SrMV. This is the first assay for differentiating strains of SCMV and SrMV that does not require interpretation of symptoms on differential hosts.

The potyvirus group is the largest and, possibly, economically most important group of the plant viruses, and sugarcane mosaic virus (SCMV) is a definitive member of this group (10,13,20). SCMV occurs in many parts of the world where susceptible species are grown and it causes mosaic disease and yield loss in sugarcane, maize, sorghum, and other poaceous plants. The SCMV complex has been shown to consist of four distinct potyviruses (18) and includes strains of Johnsongrass mosaic virus (JGMV), maize dwarf mosaic virus (MDMV), sorghum mosaic virus (SrMV), and SCMV. In sugarcane, strains of SCMV and SrMV are the most widespread viruses of this crop, and they can reduce sugar yields significantly. In the mid-1920s, disease epidemics caused by members of the SCMV subgroup accounted for a near collapse of the industry in Argentina, Brazil, and Louisiana. Although these epidemics were brought under control with the introduction of resistant sugarcane cultivars, strains of SCMV and SrMV continue to be a potential threat to the industry (7).

In the United States, 21 potyviruses belonging to the SCMV subgroup have been reported (16), and, of these, five strains of SCMV and three strains of SrMV are currently present in sugarcane fields throughout the world (7). Historically, isolates first found in sugarcane were designated as strains of SCMV (1) and those found in maize as strains of MDMV (9). However, strains of SCMV and MDMV were found to share many common properties and, therefore, MDMV was considered to be a strain of SCMV (13). Recent taxonomic research (8,11,16) on a large number of strains of viruses in the SCMV complex, based on biochemical properties of coat proteins, led to the conclusion that the complex is actually

comprised of four distinct potyviruses, and the addition of the SrMV and JGMV nomenclature and grouping has been adopted (16,18). To date, however, the various biochemical and serological techniques used for taxonomic separation cannot distinguish the strains of SCMV and SrMV (16) and, therefore, host reaction remains the only method to distinguish individual strains (6).

To investigate these relationships at the molecular level, develop a rapid, nucleic acid-based method for strain discrimination, and assist us in our design of coat protein constructs to be used in a transgenic approach for control of these viruses, we have established a collection of the prominent SCMV and SrMV strains currently causing disease in sugarcane throughout the world and originally found in sugarcane in the United States. This includes SCMV strains A, B, D, and E, and the SrMV strains SCH, SCI, and SCM. In this study, cloned cDNAs representing about 2.0 kb of the 3' termini, obtained after reverse-transcriptase–polymerase chain reaction (RT-PCR) on purified virions using an oligo(dT) primer and degenerate primers with sequences located in the N1b gene, have been sequenced for each of these strains.

MATERIAL AND METHODS

Virus isolates and purification. The SCMV strains used in this study were SCMV-A, SCMV-B, SCMV-D, SCMV-E, SrMV-SCH, SrMV-SCI, and SrMV-SCM. The SCMV strains A, B, and D, and the SrMV strains SCI and SCM were obtained from the culture collection maintained by M. Grisham (USDA Sugarcane Research Unit, Houma, LA). The SCMV-E isolate was obtained from M. Irey (US Sugar Corporation, Clewiston, FL). The SrMV-SCH isolate was isolated from a field plot of NCo-310 grown in southern Texas and will be referred to as SrMV-SCH (TX). Total RNA purified (as described below) from plants infected with unknown field isolates collected in Florida and Africa was provided

Corresponding author: T. E. Mirkov; E-mail address: e.mirkov@tamu.edu

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by P. Rott (CIRAD, Montpellier, France). All strains were inoculated on *Sorghum bicolor* cv. Rio, and virions were purified as described previously (22).

Cloning and sequencing strategy. To obtain cDNAs that represented about 2.0 kb of the 3'-terminal end of the genome for each of the seven strains, we utilized a one-tube RT-PCR strategy that was performed directly on purified virions. The design of the forward primers was based on amino acid motifs in the carboxy-terminal region of the Nib gene that are highly conserved among potyviruses (3) (Table 1). One to 10 µg of purified virions, 0.5 µg of the forward primer, and 0.5 µg of the reverse primer, oligo(dT)₂₄N, in a 20-µl final volume were incubated at 55°C for 5 min. Virions and primers were then added to a RT-PCR reaction mix (100-µl final volume) that consisted of 20 mM Tris, pH 9.0, 50 mM KCl, 2.5 mM MgCl₂, 0.1% Triton X-100, 10 mM dithiothreitol (DTT), 0.2 mM each deoxynucleoside triphosphate (dNTP), 20 units of RNasin (Promega Corp., Madison, WI), 15 units of avian myeloblastosis virus reverse transcriptase (Promega Corp.), and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Norwalk, CT). Reverse transcription and PCR were performed in a Perkin-Elmer Cetus 480 thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) programmed to give one cycle at 42°C (60 min); two cycles at 94°C (5 min), 37°C (3 min), and 72°C (3 min); 35 cycles at 94°C (1 min), 52°C (1 min), and 72°C (1 to 3 min); and a final cycle at 72°C (7 min). A 10-µl aliquot of the RT-PCR products was analyzed by electrophoresis in a 1% agarose gel (15). Reaction products of the expected sizes were excised from the gel and eluted using a GENECLAN II Kit (BIO 101 Inc., La Jolla, CA), and the DNA fragments were cloned directly into pCRII (Invitrogen Corp., San Diego, CA). Recombinant plasmids were analyzed for appropriate size inserts by cleavage with *EcoRI*. For sequencing, plasmids with inserts of the expected size were amplified in *Escherichia coli* and purified using a Qiagen plasmid purification kit (Qiagen, Inc., Chatsworth, CA).

The cDNAs were sequenced by the Sequenase 2.0 dideoxy chain termination method (United States Biochemical Corp., Cleveland) as detailed by the manufacturer using external T7 and SP6 primers, or internal oligonucleotides based on the previously determined sequence. Both strands of at least two independent clones were sequenced (Fig. 1). A third clone was sequenced when there was a disagreement between the sequences of the first two clones (Table 2). Nucleotide and amino acid sequences were analyzed with the Genetics Computer Group (GCG) sequence analysis software from the University of Wisconsin (4) and Clustal W (21). Phylogenetic trees were constructed by the neighbor-joining method, and the statistical significance of branch order was estimated by 1,000 replications of bootstrap resampling of the original align-

ments by Clustal W (21). The Clustal W parameter that allows alignment positions where any of the sequences have a gap to be ignored was used for construction of the trees.

Diagnostic restriction fragment length polymorphisms (RFLPs). Based on multiple sequence alignments of our new sequences and published sequences for six other members of the SCMV subgroup, two group-specific primer pairs were designed: primer pair SCMV F3 and R3 for SCMV strains, and primer pair SrMV F3 and R3 for SrMV strains (Table 1). Total RNA was extracted and purified from 150 mg of systemically infected sorghum or sugarcane leaves using Plant RNeasy Total RNA columns (Qiagen, Inc.) and eluted in a final volume of 50 µl of diethylpyrocarbonate-treated water. Four microliters of the eluted RNA, 3 µl of a 2.5 mM dNTP mix, 3 µl of 5× first-strand buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, and 15 mM MgCl₂), 1 µg of random primers (random hexadeoxyribonucleotides; Promega Corp.), and 2 µl of H₂O were incubated at 65°C for 3 min, and then transferred to ice for 3 min. For the reverse transcription step, 0.5 µl of 100 mM DTT, 40 units of RNasin (Promega Corp.), and 200 units

Fig. 1. Schematic representation of the 3'-terminal region of the sugarcane mosaic potyvirus genome and the cloning strategy used in this work. Genes are presented as rectangles. Nib = putative RNA-dependent RNA polymerase, CP = coat protein, and 3' UTR = 3'-untranslated region. Arrows represent position of cloning primers with the corresponding primer designation (Table 1). Horizontal bars represent clones used for sequencing, with the respective strains indicated to the left.

TABLE 1. Primers used for cloning and reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis

Primer designation	Sequence ^a
Cloning primers	
Forward primers	
pot2	TGYGAYGCNGATGGYTC
sc1	CHATWTTGGARTGGGAYG
sc5	TGYGATGCMGATGGTTCRCAA
Reverse primers	
sc2	ATTCCACCATTGCAGCAC
sc3	ATGGGAAGACTGTTTGTT
sc4	CTCTGTTCGAGTTCGGATACTC
(dT)	(dT) ₂₄ N
Primers for RT-PCR restriction fragment length polymorphism analysis	
SCMV F3	TTYCACCAAGCTGGAA
SCMV R3	AGCTGTGTCTCTCTGTATTCTC
SrMV F3	AAGCAACAGCACAAAGCAC
SrMV R3	TGACTCTACCGACATTCC

^a Redundancy code: N = A + C + T + G, M = A + C, R = A + G, Y = C + T, W = A + T, and H = T + C + A.

TABLE 2. Nucleotide differences between two independent clones that result in amino acid changes

Strain	Nucleotide ^a position	Region ^b	Nucleotide difference	Amino acid change
SCMV-A	749, 750	Nib	GT-AA	Gly-Glu
SCMV-B	905	CP	T-C	Val-Ala
	991	CP	A-G	Ser-Gly
	1,340	CP	A-G	Glu-Gly
	1,465	CP	G-A	Glu-Lys
	1,487	CP	G-A	Arg-Gln
	1,619	CP	T-C	Val-Ala
SCMV-E	1,036	CP	A-G	Ile-Val
SrMV-SCI	884	CP	C-T	Ala-Val
	898	CP	A-G	Thr-Ala
	1,148	CP	G-A	Arg-Lys
SrMV-SCM	264	Nib	T-G	Phe-Leu

^a Numbering begins with the 5'-terminal nucleotide of the clones generated in this work as indicated in Figure 1.

^b CP = coat protein.

of SuperScript II reverse transcriptase (Gibco BRL, Gaithersburg, MD) were added to the above mixture and incubated at 37°C for 60 min. Two microliters of the reverse transcription products was used as a template for PCR. The PCR reaction mix (100-µl final volume) consisted of 250 ng of each primer, 60 mM Tris-HCl (pH 8.5), 75 mM (NH₄)₂SO₄, and 2 mM MgCl₂. The cycling parameters were one cycle at 94°C (2 min); 40 cycles at 94°C (1 min),

60°C (1 min), and 72°C (1 min); and a final cycle at 72°C for 7 min. The amplified products were precipitated from the aqueous phase by addition of sodium acetate to 0.3 M and 2.5 volumes of ethanol. The pellets were washed with 70% ethanol two times and dissolved in 20 µl of distilled water. The PCR products generated from SCMV strains were digested with *TaqI*, or *HinfI*, or *ApoI* and *XbaI*. The PCR products generated from SrMV strains were

TABLE 3. Percent amino acid sequence identity between the coat protein genes and Nib genes from strains of viruses in the sugarcane mosaic virus (SCMV) subgroup^{a,b}

	JGMV-MDKS1	JGMV-MDO	JGMV-JG	SCMV-A	SCMV-E	SCMV-B	SCMV-D	SCMV-SC	SCMV-MDB	SrMV-SCI	SrMV-SCM	SrMV-SCH (TX)	SrMV-SCH	MDM V-A
JGMV-MDKS1		99	94	59	60	58	59	59	59	56	57	57	56	59
JGMV-MDO	100		93	59	60	58	59	59	59	56	57	57	56	59
JGMV-JG	87	84		61	62	60	60	60	60	57	57	57	55	60
SCMV-A	31	16	50		97	98	99	97	86	75	75	75	74	77
SCMV-E	33	22	50	97		95	96	95	83	75	75	75	73	76
SCMV-B	33	22	50	99	98		98	96	84	75	75	75	73	76
SCMV-D	33	22	50	98	98	100		97	84	75	75	76	73	76
SCMV-SC	33	22	35	96	98	98	98		84	73	73	73	72	76
SCMV-MDB	33	21	37	88	90	90	90	88		67	67	67	66	76
SrMV-SCI	36	28	54	77	76	77	77	69	73		99	98	96	80
SrMV-SCM	36	28	53	77	76	77	77	69	73	100		98	96	80
SrMV-SCH (TX)	36	28	53	76	75	76	76	67	71	99	99		97	80
SrMV-SCH	36	28	53	77	76	77	76	69	73	99	99	98		79
MDMV-A	28	19	47	75	75	75	75	58	58	80	79	79	79	

^a The sources of sequence data were GenBank accession numbers D00094 (JGMV-JG), U07217 (JGMV-MDO), U07218 (JGMV-MDKS1), D00948 (SCMV-SC), U07216 (MDMV-A), D00947 (SCMV-MDB), and U07219 (SrMV-SCH). The sequences determined in this study are GenBank accession numbers U57354 (SCMV-A), U57355 (SCMV-B), U57356 (SCMV-D), U57357 (SCMV-E), U57358 (SrMV-SCH TX), U57359 (SrMV-SCI), and U57360 (SrMV-SCM). Sequence identities were calculated by making pairwise comparisons using the Gap program (University of Wisconsin Genetics Computer Group, Madison).
^b Comparisons above the diagonal refer to the coat protein genes and comparisons below the diagonal refer to partial sequences of the Nib genes.

JGMV-MDKS1	-10	sEDvLEvEHQ	sGne..DAGR	QksaapAenK	Pasgegkpaq	ttAtsdnkps	sdtntstQGT	sqaKGdsEsG	GtkastAt..					110
JGMV-MDO		sEDvLEvEHQ	sGne..DAGR	QksaapAenK	Pasgegkpaq	ttAtsdnkps	sdtntstQGT	sqaKGdsEsG	GtkastAt..					KDK
JGMV-JG		sEDvLEvEHQ	sGne..DAGR	QksaapAenK	Pasgegkpaq	ttAtsdnkps	sdtntstQGT	sqaKGdsEsG	GtkastAt..					KDK
SCMV-B		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..TTGGQrDK
SCMV-D		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..VTGGQrDK
SCMV-A		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..VTGGQrDK
SCMV-E		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..VTGGQrDK
SCMV-SC		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..VTGGQrDK
SCMV-MDB		EDyNeEVFHQ	AG..TVDAGA	GGGGgNAGTQ	PPATGAAVQG	gagPpatGAA	AQPPaa.Q..	..ptGgatGG	GsAgTGAgGt	gS.....				..VTGGQrDK
SrMV-SCI		EDeLiDvRHQ	AGGGTVDAGA	ataeatAqaQ	rdAaakAqrd	adAkkkadde	AaerqQdaa	aKkKaddDak	aKadadAKaK	S.....				..TKGQrDr
SrMV-SCM		EDeLiDvRHQ	AGGGTVDAGA	ataeatAqaQ	rdAaakAqrd	adAkkkadde	AaerqQdaa	aKkKaddDak	aKadadAKaK	S.....				..TKGQrDr
SrMV-SCH TX		EDeLiDvRHQ	AGGGTVDAGA	ataeatAqaQ	rdAaakAqrd	adAkkkadde	AaerqQdaa	aKkKaddDak	aKadadAKaK	S.....				..TKGQrDr
SrMV-SCH		EDeLiDvRHQ	AGGGTVDAGA	ataeatAqaQ	rdAaakAqrd	adAkkkadde	AaerqQdaa	aKkKaddDak	aKadadAKaK	S.....				..TKGQrDr
MDMV-A		aDEiDvKHQ	AGen.VDAGG	kt.....		..daQkeae..		..kKaaEkk	aK.eaeAKqK					dg.gsigrDK
Consensus		-----V-HQ	-G----DAG-			..daQkeae..		..kKaaEkk	aK.eaeAKqK					-----D-
JGMV-MDKS1	111	DVDVGsTGTF	vIPKLKkvSp	KMRLPmvsK	aILnLDhLiQ	YKpQrDISN	aRAThtqFqf	WYnrIKKEYD	VDDeQmRILM	nGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	230
JGMV-MDO		DVDVGsTGTF	vIPKLKkvSp	KMRLPmvsK	aILnLDhLiQ	YKpQrDISN	aRAThtqFqf	WYnrIKKEYD	VDDeQmRILM	nGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
JGMV-JG		DVDVGsTGTF	vIPKLKkvSp	KMRLPmvsK	aILnLDhLiQ	YKpQrDISN	aRAThtqFqf	WYnrIKKEYD	VDDeQmRILM	nGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-B		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-D		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-A		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-E		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-SC		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SCMV-MDB		DVDAGTTGKI	TVPKLKAMSK	KMRLPKAKGK	DVLHLDLFLIT	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SrMV-SCI		DVDVGTSgTV	aVPLKAMSK	KmKLpQAGK	nILHLDLFLG	YKpQQQDISN	TRATREEDFR	WYDAIRKKEYE	IDDTQMTVVA	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SrMV-SCM		DVDVGTSgTV	aVPLKAMSK	KmKLpQAGK	nILHLDLFLG	YKpQQQDISN	TRATREEDFR	WYDAIRKKEYE	IDDTQMTVVA	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SrMV-SCH TX		DVDVGTSgTV	aVPLKAMSK	KmKLpQAGK	nILHLDLFLG	YKpQQQDISN	TRATREEDFR	WYDAIRKKEYE	IDDTQMTVVA	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
SrMV-SCH		DVDVGTSgTV	aVPLKAMSK	KmKLpQAGK	nILHLDLFLG	YKpQQQDISN	TRATREEDFR	WYDAIRKKEYE	IDDTQMTVVA	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
MDMV-A		DVDAGTSgSv	sVPLKAMSK	KMRLPqAGK	nILHLDLFLK	YKpQQQDISN	TRATREEDFR	WYEAIRKKEYE	IDDTQMTVVM	SGLMVWCIE	GtSPdINGyW	TmVDGnnQse	FPLKPIVENA	
Consensus		DVD-G -G- -	--P-LK--S-	KM-LP----	--L-LD-L-	YKp-Q-D-SN	--RAT--F--	WY---KEY-	--DD-QM----	--GLMVW-IE	G-SP-I---W	TM-D---Q--	FPLKPIVENA	B-EA
JGMV-MDKS1	231	kPTLRQcMmH	FSDAEAAYIE	mRNlGEpYMP	RYGLLRNLnD	kSLARYAFDF	YEINSRTPhr	ArEAHaQMKA	AAIRGSntnm	FGLDGNVGS	sENTERHTAA	DVSRNVHSyr	Gaki..*	347
JGMV-MDO		kPTLRQcMmH	FSDAEAAYIE	mRNlGEpYMP	RYGLLRNLnD	kSLARYAFDF	YEINSRTPhr	ArEAHaQMKA	AAIRGSntnm	FGLDGNVGS	sENTERHTAA	DVSRNVHSyr	Gaki..*	
JGMV-JG		kPTLRQcMmH	FSDAEAAYIE	mRNlGEpYMP	RYGLLRNLnD	kSLARYAFDF	YEINSRTPhr	ArEAHaQMKA	AAIRGSntnm	FGLDGNVGS	sENTERHTAA	DVSRNVHSyr	Gaki..*	
SCMV-B		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SCMV-D		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SCMV-A		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SCMV-E		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SCMV-SC		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SCMV-MDB		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SrMV-SCI		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SrMV-SCM		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SrMV-SCH TX		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
SrMV-SCH		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
MDMV-A		SPTFRQIMHH	FSDAEAAYIE	YRNSTERYMP	RYGLQRNLTD	YSLARYAFDF	YEMNSRTPAR	ArEAHaQMKA	AAVRGSNTRL	FGLDGNVGS	QENTERHTAG	DVSRNMHSL	GVQQHH*	
Consensus		-PT-RQ-M-H	FSDAEAAYIE	-RN--E-YMP	RY-L-RN--D	--LARYAFDF	YE--SRTP-	--BAH-QMKA	-A--GS----	--GLDGNVGS	-ENTERHTA-	D-S-N-HS--	G-----*	

Fig. 2. Multiple alignment of deduced amino acid sequences of coat protein genes from 13 members in the sugarcane mosaic potyvirus subgroup. The alignment was generated using the PileUp and Pretty programs (University of Wisconsin Genetics Computer Group, Madison). Seven of these sequences have already been published; sequences of new strains are indicated in bold. The arrow indicates the putative cleavage site between the Nib gene product and coat protein. Duplicated peptide sequences are underlined. Consensus amino acids are given as upper case, and the Consensus line shows only amino acids that are identical for all 14 members.

digested with *Hga*I or *Taq*I and *Sau*3AI. The digestion products were analyzed by electrophoresis through a 1.7% agarose gel or a 6% polyacrylamide gel (14).

RESULTS

Cloning and sequencing strategy. Clones representing approximately 2.0 kb of the 3'-terminal region of the viral genome were obtained in one step for the SCMV strains B, D, and E. This was achieved using the forward primer pot2 and an oligo(dT) primer as the reverse primer (Table 1, Fig. 1). The pot2 primer is a degenerate primer coding for the amino acid sequence CDADGS, which is a highly conserved motif in the N1b genes of potyviruses (3). For strains SCMV-A, SrMV-SCH, SrMV-SCI, and SrMV-SCM, clones representing approximately 2.0 kb of the 3'-terminal region of the viral genome were obtained in two steps. First, primer sc1 and an oligo(dT) primer (Table 1, Fig. 1) were used to amplify the 3' end of the N1b gene, the coat protein gene, and the 3'-untranslated region. The degenerate sc1 primer was based on a conserved amino acid motif (SILEWDR) identified in the N1b genes of strains B, D, and E. In the second step, the upstream region of the N1b gene corresponding to that obtained for strains B, D, and E was amplified with reverse primers based on previously determined sequences in the coat protein (sc2 or sc3) or N1b genes (sc4), and forward primers pot2 or sc5 (Table 1, Fig. 1). The degenerate primer sc5 is a modification of pot2 that was based on sequences obtained from clones of strains B, D, and E.

The clones obtained for the seven SCMV isolates used in this study contain a short poly(A) tract, the 3'-untranslated region of the viral genome, the sequence encoding the complete coat protein, and the 3' half of the N1b gene. In all cases, there were nucleotide differences between the first two independent clones that were sequenced. In the majority of instances, the differences were in the third position of the codon and did not result in an amino acid change. The differences that resulted in an amino acid change are given in Table 2. These differences were confirmed by sequencing a third clone. In no cases was there evidence that the differences were due to errors during PCR amplification, hence, these differences reflect on the quasispecies nature of the populations. The complete sequences for all clones corresponding to approximately 2.0 kb of the 3'-terminal region of the genomes of the four SCMV and three SrMV strains used in this study have been deposited with the GenBank data library under accession numbers U57354 (SCMV-A), U57355 (SCMV-B), U57356 (SCMV-D), U57357 (SCMV-E), U57358 (SrMV-SCH), U57359 (SrMV-SCI), and U57360 (SrMV-SCM) and are not presented here as a separate figure.

Partial sequences and comparisons of the N1b genes. The partial sequence of the 3' half of the N1b gene was 276 amino acids in length for all seven isolates. The amino acid identity was from 98 to 100% within the SrMV group, and between 96 to 100% within the SCMV group with the exception that SCMV-MDB was only 88 to 90% identical (Table 3). The amino acid identity between the two groups averaged about 76% (Table 3).

Sequences and comparisons of the coat protein genes. A multiple sequence alignment of the deduced coat protein and immediate upstream sequences of the seven isolates sequenced in this study, and the sequences of another seven members of the SCMV group for which the sequences are available from GenBank, is presented in Figure 2. The putative hydrolytic cleavage site, glutamine/serine or glutamine/alanine, between the replicase and the coat protein is indicated by the arrow. The amino acids surrounding these predicted cleavage sites conform to the consensus sequence recognized by the N1a proteinase responsible for cleavage of the coat protein from the viral polyprotein (15). The lengths of the SCMV and SrMV coat proteins varied from 305 to 329 residues. All strains of SrMV were 329 residues, SCMV-A was 304 residues, SCMV-B and -D were 309 residues, SCMV-E and -SC were 313 residues, and SCMV-MDB was 328 residues.

The amino acid identity was from 98 to 100% within the SrMV group, and between 96 to 100% within the SCMV group with the exception that SCMV-MDB was only 83 to 86% identical (Table 3). The amino acid identity between the two groups averaged about 75% (Table 3). The phylogenetic relationships of the conserved core (Fig. 2, residues 109 to 347) of the coat proteins (Fig. 3) among 14 members of the SCMV subgroup were examined using the Clustal W programs (21). In this analysis, SCMV-MDB groups with the rest of the SCMV strains. However, if the entire coat protein sequence was used, using the Clustal W parameter that allows alignment positions where any of the sequences have a gap to be ignored (21), SCMV-MDB did not group with the rest of the SCMV strains (Fig. 3).

Fig. 3. Phylogenetic trees for the conserved core of the coat protein (residues 109 to 347 in Fig. 2) (top), and the entire coat protein (bottom). Trees were constructed by the neighbor-joining method with 1,000 bootstraps and the parameter that ignores alignment positions where any of the sequences have a gap (Clustal W). The number beside each node indicates the number of bootstrap replicates.

One feature in the N-terminal region of the coat proteins shared by all 14 members is the conserved DAG motif (Fig. 2), shown to be associated with aphid transmissibility (2). Another interesting feature in the N-terminal region of the coat proteins of these new sequences is the short repeated peptide motifs, similar to those first observed for SCM-V-SC by Frenkel et al. (5) and Xiao et al. (24). The sequence pattern GAQPPATGAAAQ (underlined in Fig. 2) was repeated nearly perfectly in the SCM-V strains A, B, D, E, and SC. These five strains also contained a third partial repeat of PPA/TA/T. This was in contrast to the imperfect repeat motif of AGSGSGTGAGTGVTGGQ (underlined in Fig. 2) found in the

MDV strain of SCM-V (5,24). The three SrMV strains contained a third distinct pattern of DADAKKKADDEAA (underlined in Fig. 2) that was repeated exactly. A third nearly perfect repeat of this sequence was located between the two perfect repeats (Fig. 2). No repeated sequences were identified in the JGMV strains or in MDMV-A.

Sequences and comparisons of the 3'-untranslated regions. The 3'-untranslated regions varied in length from 230 bases for the SCM-V strains to 234 bases for the SrMV strains. The nucleotide sequence identity was from 97 to 100% within the SrMV group, and between 95 to 98% within the SCM-V group with the

TABLE 4. Percent nucleotide sequence identity between the 3'-noncoding regions from strains of viruses in the sugarcane mosaic virus (SCMV) subgroup^a

	JGMV-MDO	JGMV-JG	SCMV-A	SCMV-E	SCMV-D	SCMV-B	SCMV-SC	SCMV-MDB	SrMV-SCI	SrMV-SCM	SrMV-SCH (TX)	SrMV-SCH	MDMV-A
JGMV-MDKS1	99	93	46	43	44	47	45	38	40	40	39	40	41
JGMV-MDO		92	45	43	44	47	45	38	39	39	35	39	41
JGMV-JG			44	44	43	44	46	40	36	36	41	36	41
SCMV-A				98	97	98	97	90	60	60	60	60	55
SCMV-E					97	96	95	89	60	61	60	60	55
SCMV-D						98	95	90	60	60	60	60	57
SCMV-B							95	89	60	60	60	60	55
SCMV-SC								88	59	59	59	59	52
SCMV-MDB									60	54	54	59	54
SrMV-SCI										99	99	100	61
SrMV-SCM											99	99	61
SrMV-SCH (TX)												97	60
SrMV-SCH													61

^a The sources of sequence data and calculations of sequence identities were as in Table 3.

JGMV-MDKS1	1	GGAGGAGGAA	CAACCGTCTA	TCTGACGTTA	AAGGATGACT	GGCCAACTCT	GAAAAATGGC	GTGATACCAG	TTCCGAATAG	TCCATACAGA	ATTAGAGGTT	AACAGAGAGC	ACTATAAAGG	120
JGMV-MDO		GGAGGAGGAA	CAACCGTCTA	TCTGACGTTA	AAGGATGACT	GGCCAACTCT	GAAAAATGGC	GTGATACCAG	TTCCGAATAG	TCCATACAGA	ATTAGAGGTT	AACAGAGAGC	ACTATAAAGG	
JGMV-JG		GGAGGAGGAA	CAACCGTCTA	TCTGACGTTA	AAGGATGACT	GGCCAACTCT	GAAAAATGGC	GATATACCAG	TTCCGAATAG	TCCGTACAGA	ATTAGAGGTT	AACAGAGAGC	ACTATAAAGG	
JGMV-MDKS1	121	TTTAGTAGGG	ATCGAGAGAT	GACACGTAGG	ACCTCAATGA	CCATGCTTAG	CGCTAGCAAT	GTGTGGAGCT	CTAACACTCA	GgTGTtatct	tcCaggCTgT	GgtaaatCTcg	TAccccGaAg	240
JGMV-MDO		TTTAGTAGGG	ATCGAGAGAT	GACACGTAGG	ACCTCAATGA	CCATGCTTAG	CGCTAGCAAT	GTGTGGAGCT	CTAACACTCA	GgTGTtatct	tcCaggCTgT	GgtaaatCTcg	TAccccGaAg	
JGMV-JG		TTTAGTAGGG	ATCGAGGAT	GACACGTAGG	ACCTCAATGA	CCATGCTTAG	CGCTAGCAAT	GTGTGAGCT	CTAACACTCA	GgTGTtatct	tcCaggCTgT	GgtaaatCTcg	TAccccGaAg	
SCMV-B														
SCMV-D														
SCMV-A														
SCMV-E														
SCMV-SC														
SCMV-MDB														
SrMV-SCI														
SrMV-SCH														
SrMV-SCM														
SrMV-SCH TX														
MDMV-A														
Consensus														
JGMV-MDKS1	241	TAccTATggg	CccTcagcac	cAGacagTAC	tggGactaTg	TatCcTTTcT	tGcAaTcAC	TagtATtaTa	AAcCgTTCTG	CAatACACct	GGgTGAaTc	cGgcaCgagT	GGTgttTcTa	360
JGMV-MDO		TAccTATggg	CccTcagcac	cAGacagTAC	tggGactaTg	TatCcTTTcT	tGcAaTcAC	TagtATtaTa	AAcCgTTCTG	CAatACACct	GGgTGAaTc	cGgcaCgagT	GGTgttTcTa	
JGMV-JG		TAccTATggg	CccTcagcac	cAGacagTAC	tggGactaTg	TatCcTTTcT	tGcAaTcAC	TagtATtaTa	AAcCgTTCTG	CAatACACct	GGgTGAaTc	cGgcaCgagT	GGTgttTcTa	
SCMV-B		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SCMV-D		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SCMV-A		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SCMV-E		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SCMV-SC		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SCMV-MDB		TA...ATAat	gTgTaTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SrMV-SCI		TATATATtta	CTATTTTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SrMV-SCH		TATATATtta	CTATTTTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SrMV-SCM		TATATATtta	CTATTTTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
SrMV-SCH TX		TATATATtta	CTATTTTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
MDMV-A		TATATATtta	CTATTTTCAGT	GAGGTTCTAC	CTCG. TcTc.	.TACTaTaTT	ACGTATGTAC	TtAAAGCGTG	AACCAgTCTG	CAGGACACAG	GgTGGACCC	agTgtCTTCT	GGT. .GTAGc	
Consensus		TA---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	---T---T---T	
JGMV-MDKS1	361	aTgctgTtat	acaGttatgg	aTACGGcCc	GgtgTGTtG	TatTTactag	tTGcaTCAGA	ccaTctGAGc	CTCagaGTGA	GcttCTCACC	ACGCAGTCTC	TTATGGCGAG	ATA. .	475
JGMV-MDO		aTgctgTtat	acaGttatgg	aTACGGcCc	GgtgTGTtG	TatTTactag	tTGcaTCAGA	ccaTctGAGc	CTCagaGTGA	GcttCTCACC	ACGCAGTCTC	TTATGGCGAG	ATA. .	
JGMV-JG		aTgctgTtat	acaGttatgg	aTACGGcCc	GgtgTGTtG	TatTTactag	tTGcaTCAGA	ccaTctGAGc	CTCagaGTGA	GcttCTCACC	ACGCAGTCTC	TTATGGCGAG	ATA. .	
SCMV-B		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SCMV-D		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SCMV-A		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SCMV-E		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SCMV-SC		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SCMV-MDB		GTGTACTAGC	GTGACGcAc	gTACCGGACA	GcGcTgGTG	TGcTTTGCC	ATtgGT. .	.GCTGCGAGT	CTCtTgGTGA	GAGAC. .				
SrMV-SCI		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				
SrMV-SCH		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				
SrMV-SCM		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				
SrMV-SCH TX		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				
MDMV-A		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				
Consensus		tcctTACTAGC	GTGAAcTcAc	aTtACGGACA	cCctgtTGTG	TGgTtTcTACC	AcGAGTCAGg	AGtTGCAGGT	aTtgTaGcaA	GAGAC. .				

Fig. 4. Multiple alignment of nucleotide sequences in the 3'-untranslated region from 13 members in the sugarcane mosaic potyvirus subgroup. The alignment was generated using the PileUp and Pretty programs (University of Wisconsin Genetics Computer Group, Madison). Seven of these sequences have already been published; sequences of new strains are indicated in bold. Consensus nucleotides are given as upper case, and the Consensus line shows only nucleotides that are identical for all 14 members.

could be generated with *TaqI*, *Sau3AI*, and *HinfI*. These restriction enzymes digest the amplification products at three or four sites (Fig. 8).

An example of the identification of unknown field isolates is given in the bottom panel of Figure 8. The five unknowns were first determined to be strains of SCMV as only the SCMV-specific primer pair primed the amplification of RT-PCR products (data not shown). Digestion of the RT-PCR products with *TaqI* revealed that isolates 2 and 5 were SCMV strain A, and that isolate 1 was SCMV strain B (Fig. 8). The *TaqI* RFLP could not distinguish whether isolates 3 and 4 were SCMV strain D or E. Further analysis with *HinfI* and *XbaI* digestions showed that both isolates 3 and 4 were SCMV strain E (data not shown).

DISCUSSION

Until recently, it was well recognized that the taxonomy of the potyviruses was in a "very unsatisfactory state" (23). However, the continuous accumulation and analysis of potyvirus genomic sequence data appears to have overcome these problems. Shukla and Ward (17) and Ward et al. (23) have demonstrated that coat protein gene sequence data can be used to provide a hierarchical classification of potyviruses. They found that pairwise coat protein amino acid sequence comparisons among 17 strains of eight distinct potyviruses showed a bimodal distribution. Distinct viruses of the group exhibited sequence identities from 38 to 71%, whereas strains of the same virus exhibited sequence identities from 90 to 99%. Additional sequence data (12) have identified a

third cluster in the 75 to 89% range. Shukla and Ward (17) have proposed that viruses that fall into this group should be considered distinct species rather than strains of the same virus.

We applied the criteria of Shukla and Ward (17) to our comparative sequence analysis of the seven viruses sequenced in this study and six other members of the SCMV subgroup for which sequence data were available in GenBank (Tables 3 and 4). Based on their criteria, our comparative sequence analysis confirms prior biochemical analysis (8,11,16) that identified at least four distinct groups of SCMV, but suggests that there are five distinct groups. When compared with SCMV strains A, B, D, E, and SC, the identities for SCMV-MDB ranged from only 83 to 86% in the coat protein, and from 88 to 90% in the NIb gene and the 3'-untranslated region. As proposed by Shukla and Ward (Fig. 3 in literature citation 12), this is below the cutoff point for strains of the same virus and, hence, SCMV-MDB would be considered a distinct species rather than a strain of SCMV. Additional data that would argue that SCMV-MDB be considered a distinct virus, rather than a strain of SCMV, would be the lack of relatedness between the repeated peptide motifs found in the N-terminal region of the SCMV-MDB coat protein and those found in the other SCMV strains (Fig. 2). Furthermore, SCMV-MDB does not infect sugarcane. It will be of interest to determine by sequence analysis if the Australian strains of SCMV that do not infect sugarcane fall into this fifth group of the SCMV complex.

With this work, we provide the first biochemical method that can distinguish strains of SCMV and SrMV. Prior to this, the only

Fig. 7. Restriction fragment length polymorphism of reverse-transcriptase polymerase chain reaction products from strains of sugarcane mosaic potyvirus (SCMV) and sorghum mosaic virus (SrMV). Amplification products using SrMV-specific primers (Table 1) and discrimination between strains H, I, and M, with *HgaI* digests (top). Amplification products using SCMV-specific primers (Table 1) and discrimination between strains A, B, D, and E, with *ApoI* and *XbaI* digests (bottom). S, molecular weight markers from top to bottom: 2,645, 1,605, 1,198, 676, 517, 460, 396, and 350 bp.

Fig. 8. Restriction fragment length polymorphism (RFLPs) of reverse-transcriptase polymerase chain reaction products from strains of sugarcane mosaic potyvirus (SCMV) and strain determination of unknown field isolates. RFLPs of amplification products using known SCMV strains with SCMV-specific primers (Table 1) and discrimination between strains A, B, D, and E, with *TaqI* and *HinfI* digests (top). Strain determination of unknown field isolates of strains of SCMV (bottom). S, molecular weight markers from top to bottom: 2,645, 1,605, 1,198, 676, 517, 460, 396, and 350 bp.

method available to differentiate strains was based on symptom variation on a set of differential hosts developed by Abbott and Tippet (1). Smith and van de Velde (19) have developed a RT-PCR-based detection system for strains of SCMV, but their strategy was not designed to allow for strain discrimination using RFLP analysis. Our method uses two sets of group-specific oligonucleotides that prime amplification of a region of the coat protein gene that contains RFLPs. We have shown that the SCMV primers F3 and R3 (Table 1) are specific to the SCMV strains A, B, D, and E (Fig. 7). Based on sequence comparisons, these primers would result in amplification products from tissue infected with the SC strain of SCMV, but not from tissue infected with MDMV-A, MDMV-B, or strains of JGMV. Again, based on sequence comparisons, it is predicted that the SrMV-specific primers would not result in PCR products from tissue infected with MDMV-A, MDMV-B, or strains of JGMV, and we have demonstrated that they will not result in PCR products from tissue infected with strains of SCMV. This method should facilitate rapid identification and discrimination of strains from unknown field isolates (Fig. 8).

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