Confirmation that Fourteen Potyvirus Isolates from Soybean Are Strains of One Virus by Comparing Coat Protein Peptide Profiles

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ABSTRACT

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A number of potyvirus isolates have been identified as strains of soybean mosaic virus (SMV) on the basis of host range, symptomatology, vector specificity, and antigenic properties. Comparison of recently established coat protein gene sequences of two of the strains, SMV-N and SMV-VA, suggested that they represent two distinct potyviruses. The taxonomic status of other strains relative to these two strains is uncertain at present. To address this question we have compared high-performance liquid chromatographic peptide profiles of coat protein tryptic digests from 14 such strains, including SMV-N and SMV-VA. Our results, including amino acid composition of some peptides, show that these 14 strains are all related to SMV-N and that no evidence could be found for the reported coat protein sequence of SMV-VA, implying that the sequenced SMV-VA clone was a minor contaminant of the original SMV-VA isolate. Our results also confirm that SMV-N and watermelon mosaic virus 2 (WMV 2) are closely related, and the question of whether WMV 2 is a distinct virus or an SMV pathotype is discussed.

Soybean mosaic virus (SMV), a definitive member of the potyvirus group (21), induces a variety of cultivar-dependent symptoms in soybeans, such as mild mosaic, mottling, necrosis, stunting, and bud blight (3–5,30). The seedborne (3) virus occurs worldwide and is a major limiting factor for soybean (*Glycine max* (L.) Merrill) production wherever the crop is cultivated (37,40). The virus is readily transmitted by mechanical means and also by many aphid species in a nonpersistent manner (3). SMV particles are flexuous filaments, approximately 750 nm long (3), and consist of a single species of protein, molecular weight 29,900, and a single-stranded positive-sense RNA molecule of about 10,000 bases (7).

Numerous SMV strains have been identified that vary in symptom induction, vector transmission, and antigenic properties. Based on virulence studies, Cho and Goodman (5) identified seven distinct strains (G1–G7) from 98 isolates in the USDA soybean germplasm collection. They also suspected that the necrotic strain of SMV occurring in Korea (4) was similar to their G5 strain. Based on serology and symptomatology in cowpea, bean, and pea and on reaction of soybean differential cultivars, Hunst and Tolin (18) identified two strains, SMV-VA and SMV-OCM, as belonging to the G1 and G3 strain groups, respectively, of Cho and Goodman (5). Lucas and Hill (20) distinguished three other SMV strains, 75-16-1, 12-18, and 0, on the basis of host reactions and aphid transmissibility, but their relationships to the strains G1–G7 (5) were not determined. Studies of antigenic properties (17) have shown that SMV strains can be divided into three serological groups: 1) G1; 2) G2, G3, G4, G6, and G7; 3) G5, 0, 12-18, 75-16-1, and Brazil. This grouping was achieved by one-dimensional trypsin peptide maps immunoblotted with 12 monoclonal antibodies (17).

Comparison of amino acid sequence data of potyvirus coat proteins has revealed a bimodal distribution of sequence identities with distinct members displaying, in general, sequence identities of 38–71%, and strains of individual viruses showing sequence identities of more than 90% (34–36,41). The recently established coat protein gene sequences of the two SMV strains, SMV-VA (13) and SMV-N (7), suggested that they are distinct potyviruses, not related strains, since they exhibit coat protein amino acid sequence identity of only 58% (35).

Since the coat protein gene sequence data suggests that mosaic disease in soybean is caused by two distinct viruses, SMV-N and SMV-VA, the taxonomic status of the other SMV strains is uncertain. To determine if other SMV strains are actually strains of SMV-N, SMV-VA, or even a third virus, 14 such strains were compared using high-performance liquid chromatographic (HPLC) peptide profiling of their coat protein digests. This technique reflects the extent of sequence identity between two proteins (32) and has been shown to clearly distinguish between potyviruses and their strains (23–25).

MATERIALS AND METHODS

Virus strains and isolation of coat proteins. Fourteen SMV strains, namely, G1/019-2, G2/60, G3/83-2, G4/016-2, G5/32-2, G6/U427, G7/U670 (5), and the additional strains 75-16-1/G2,

12-18, 0 (20), VA/G1 (18), N/G2 (7), Brazil (17), and Wisconsin (O. W. Barnett, *unpublished*), were investigated. Confirmation of G1–G7 pathotype responses on differential cultivars (5) was done before and after virus purification. For comparison, watermelon mosaic virus 2 (WMV 2), which is closely related to SMV-N (1,9,10,42), and the W strain of papaya ringspot virus (PRSV), a distinct potyvirus (11), also were studied. SMV strains were purified according to the methods of Hill and Benner (16), Hunst and Tolin (18), or method 2 of Reddick and Barnett (29). WMV 2 was purified according to Shukla et al (33), and PRSV-W was purified according to Hammond and Lawson (15). Coat protein was isolated using formic acid as described by Shukla et al (31). In most cases only one purification of each isolate was prepared.

Enzymic digestion of coat proteins. Freeze-dried coat proteins, suspended in 0.05 M NH_4HCO_3 (500 μl/mg of coat protein) by sonication (~5 s), were digested at 37 C for 16–17 h with trypsin (Worthington, Freehold, NJ) at an enzyme/protein ratio of 1:50. Solutions were dried in a Savant Speed-Vac (Hicksville, NY), vortexed in 0.1% trifluoroacetic acid (TFA; 500 μl/mg of protein), and clarified by centrifugation at 10,000 *g* for 2 min in a bench-top centrifuge (25).

HPLC peptide profiling. Soluble peptides were separated by injecting aliquots (10–30 μl) of protein digests into a 5-μm C_{18} reversed-phase column (4.6 × 250 mm, Vydac Corp., Hesperia, CA) connected to a Perkin-Elmer LC100, series 4, liquid chromatographic system (Norwalk, CT). Components were separated in 0.1% TFA at a flow rate of 1 ml/min using a linear gradient of 0–35% acetonitrile over 60 min at 45 C. A UV-M monitor (214 nm, Pharmacia, Uppsala, Sweden) was used to monitor peptides eluting from the column, which was purged at the end of each run by applying a gradient of 35–70% acetonitrile in 0.1% TFA over 2 min, followed by reequilibration in 0.1% TFA for an additional 8 min.

Amino acid analysis of coat proteins and peptides. Coat proteins or peptide fragments were subjected to vapor-phase hydrolysis at 110 C in 5.8 M HCl containing 0.01% phenol for 20–22 h under N_2. They were analyzed on a Water's amino acid analyzer (Millipore Corp., Milford, MA) using an ion-exchange column.

RESULTS

Comparative amino acid analysis of coat proteins from 14 strains of SMV (G1–G7, 0, 12-18, 75-16-1, N, VA, Wisconsin, Brazil) showed that their compositions were very similar to each other. The composition of WMV 2, previously shown to be closely related to SMV-N (9,10,42), was similar to the compositions of the 14 SMV strains, differing only in the number of Ala, Arg, and Thr residues (data not shown).

HPLC peptide profiles of tryptic digests from the coat proteins of 14 SMV strains are shown in Figures 1 and 2. Peptide profiles from these coat proteins were nearly superimposable, except for

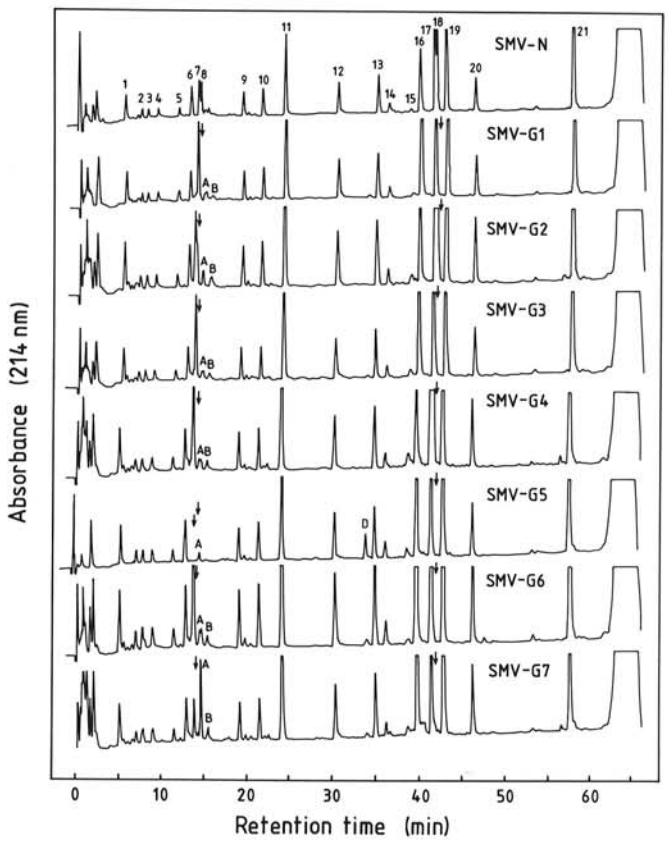

Fig. 1. High-performance liquid chromatographic peptide profiles of tryptic digests of coat proteins from eight strains of soybean mosaic virus (SMV). Dried samples were suspended in 0.05 M NH_4HCO_3, digested with trypsin, dried, and mixed with 0.1% trifluoroacetic acid (TFA). After centrifugation, the supernatant was injected onto a Vydac 5-μm C_{18} reversed-phase column (4.6 × 250 mm) connected to a Perkin-Elmer LC100, series 4, liquid chromatographic system. Peptides were eluted with a gradient of acetonitrile in 0.1% TFA at a flow rate of 1 ml/min over 60 min at 45 C. Numbered SMV-N peaks and their equivalents in other profiles were used for comparisons of profiles, together with peaks designated by one of the letters A, B, or D. Arrows denote positions where no peak was equivalent in retention time to an SMV-N peak.

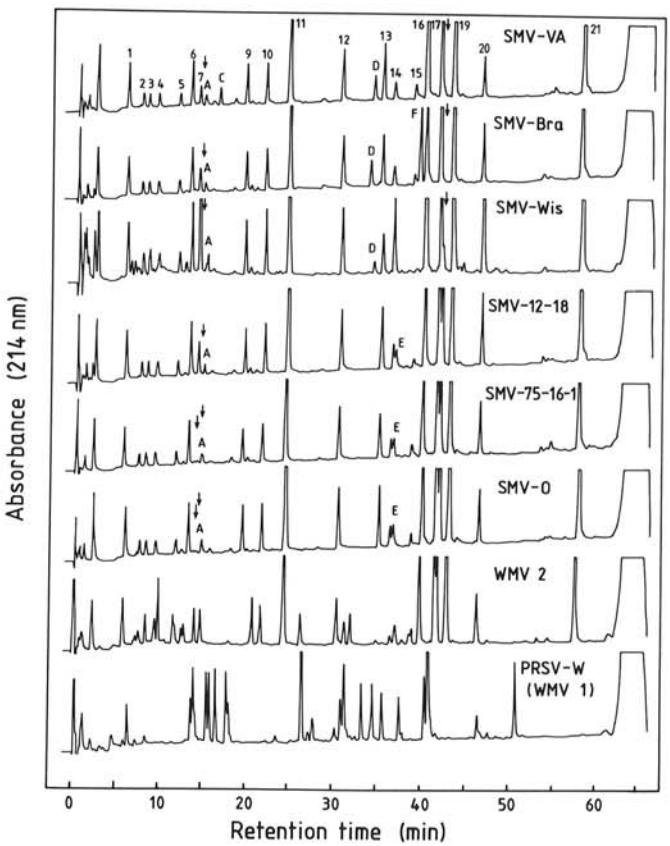

Fig. 2. High-performance liquid chromatographic peptide profiles of tryptic digests of coat proteins from the soybean mosaic virus (SMV) strains VA, Brazil (Bra), Wisconsin (Wis), 12-18, 75-16-1, and 0, together with watermelon mosaic virus 2 (WMV 2) and the W strain of papaya ringspot virus (PRSV-W), formerly known as WMV 1. Dried samples were suspended in 0.05 M NH_4HCO_3, digested with trypsin, dried, and mixed with 0.1% trifluoroacetic acid (TFA). After centrifugation, the supernatant was injected onto a Vydac 5-μm C_{18} reversed-phase column (4.6 × 250 mm) connected to a Perkin-Elmer LC100, series 4, liquid chromatographic system. Peptides were eluted with a gradient of acetonitrile in 0.1% TFA at a flow rate of 1 ml/min over 60 min at 45 C. Numbered SMV-VA peaks and their equivalents in other profiles were used for comparisons of profiles, together with peaks designated by one of the letters A, C, D, E, or F. Arrows denote positions where no peak was equivalent in retention time to an SMV-N peak.

the loss of one or two peaks and the addition of up to two other peaks in some profiles. Amino acid analysis of component peptides showed that in the overwhelming number of cases, peaks with matching retention times from different profiles also had matching amino acid compositions (Fig. 3) corresponding to the known tryptic peptides of SMV-N (7).

In the few cases where no peak corresponded to an SMV-N peak in retention time (peaks equivalent to 7, 8, and 18 of SMV-N were absent in some SMV strains), peptides were identified in the other profiles that were homologous in composition to the equivalent SMV-N peptides (Table 1). For example, peak 8 of SMV-N, corresponding to residues 6–16 of the coat protein (7), was absent from all other profiles of SMV strains (Figs. 1 and 2). However, rechromatography of peak 7 of G1 at 2 C gave two peaks (data not shown), one of which (7′) was identical in amino acid composition to that of SMV-N peak 8 (Fig. 3 and Table 1). Similarly, the absence in the G1–G7, VA, Brazil, and Wisconsin profiles of a peak with retention time equal to that of peak 18 of SMV-N (Figs. 1 and 2) was balanced by the isolation from peak 17 of two peptides, 17′ and 17″ (by rechromatography at 2 C, data not shown). The amino acid composition of the latter peptide was identical with peak 18 of SMV-N (Table 1).

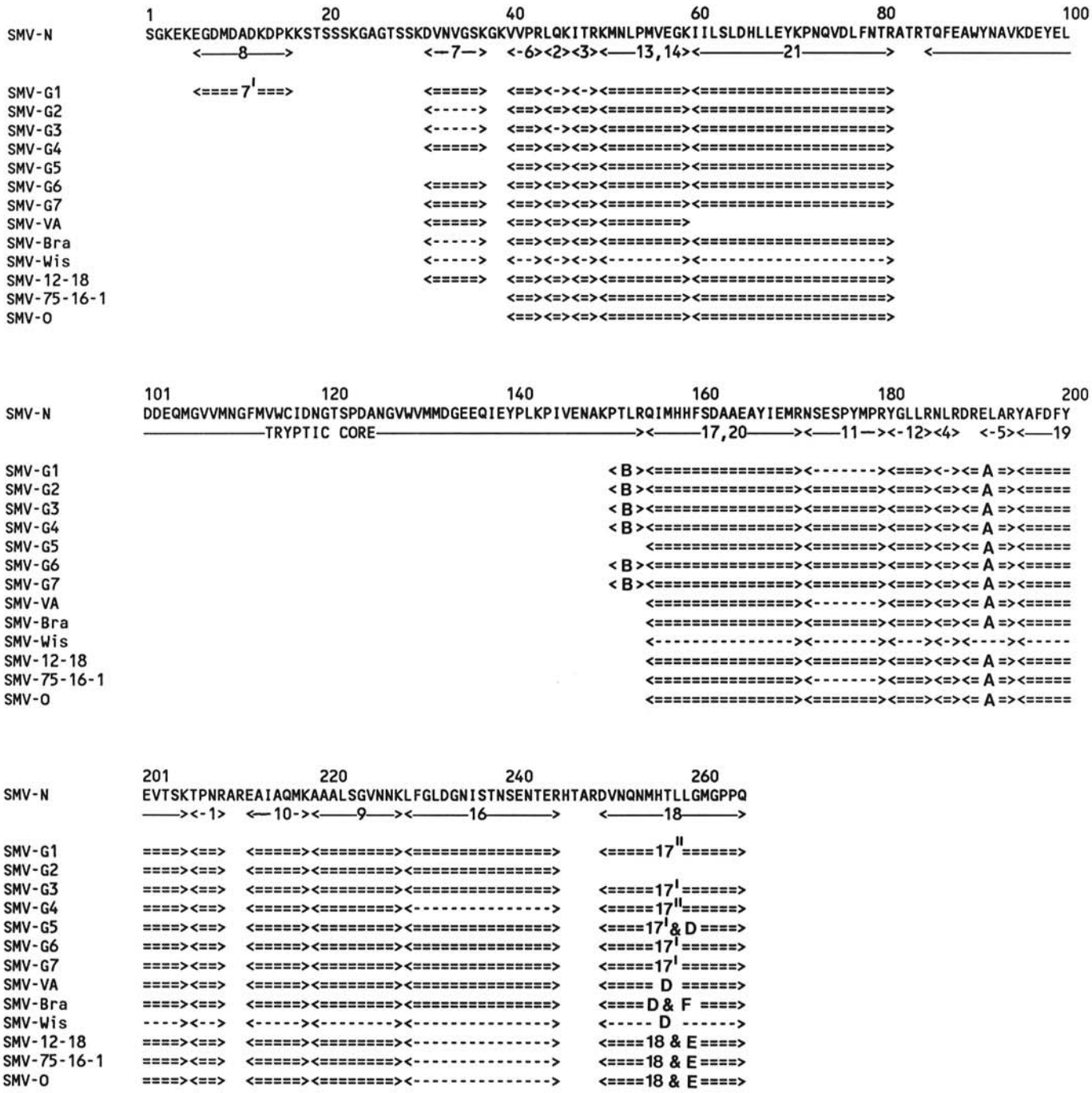

Fig. 3. Alignment of tryptic peptides from coat proteins of soybean mosaic virus (SMV) strains. SMV-N peptides shown in solid lines were numbered according to the peaks in Figure 1 from which they were obtained and were placed by amino acid composition and referral to the SMV-N coat protein sequence (7). Peptides shown in single-dashed lines were placed by retention time similarity with the SMV-N peaks. Peptides denoted by a double-dashed line were placed by retention time similarity and identical or similar amino acid composition to the SMV-N peptides. Letters denote peaks in Figures 1 and 2 with retention times not shared by SMV-N peaks. Peaks 7′, 17′, and 17″ (see Table 1) were additional peaks obtained after rerunning peaks 7 and 17, respectively, on reversed-phase high-performance liquid chromatography (data not shown).

All of the additional peaks A–F not observed in SMV-N (Figs. 1 and 2) were found to have amino acid compositions homologous to those of known peptides of SMV-N, thus enabling them to be placed within the coat protein structure of SMV-N (Fig. 3). In all cases, the apparent compositions either were identical with those of SMV-N peptide fragments or differed by only one or two amino acids (Table 1). None of the coat proteins studied, including our isolate of SMV-VA, had the peptide profiles expected from the published sequence of SMV-VA (13).

Considered overall, peptides isolated from the 21 peaks of the SMV-N profile analyzed in this study (Figs. 1 and 3), together with the region spanned by the insoluble tryptic core (residues 85 to 154, Fig. 3), accounted for all but 27 amino acids of the coat protein sequence. Homologous peptides could be found for the majority of coat proteins from the other strains of SMV, except for the insoluble tryptic core regions that were not examined. The peptide profiles of WMV 2 coat protein were found to have 17 of 25 peaks with retention times and relative heights similar to those of SMV-N peaks (Fig. 2). In contrast, only a few peaks (4 of 27) of the peptide profile of PRSV-W had retention times similar to those of SMV-N (Fig. 2).

DISCUSSION

Shukla and Ward (34–36,41) have shown that the degree of amino acid sequence identity between the coat proteins of potyviruses reflects their taxonomic relationships. Distinct members, in general, possess sequence identities of up to 71%, whereas strains of individual viruses are more than 90% identical, resulting in a bimodal distribution of sequence identities between distinct

TABLE 1. Amino acid compositions of high-performance liquid chromatographic peaks from coat protein tryptic digests of soybean mosaic virus (SMV) strains

	Peak name[a]										
			D				17				
Amino acid[b]	A[c]	B	(i)	(ii)	E	F	(i)	(ii)	17′	17″	7′
Ala	1	...	...	...	...	...	3	2 (3)	...	...	1
Arg	2	1	...	...	...	...	... (1)	1	...	...	...
Asx	1	...	3	3	3	3	1	1	3	3	3 (4)
Glx	1	...	3 (2)	3 (2)	3 (2)	3 (2)	4 (3)	4 (3)	3 (2)	2	1
Gly	...	...	2	2	2	2	...	...	2	2	2 (1)
His	...	...	1	1	1	1	2	2	1	1	...
Ile	...	...	...	...	...	...	2	2	...	...	...
Leu	1	1	2	2	2	2	...	...	2	2	...
Lys	...	...	...	...	...	...	...	...	...	...	2
Met	...	...	2	2	2	2	2	2	2	2	1
Phe	...	...	...	...	...	...	1	1	...	...	...
Pro	...	1	1 (2)	1 (2)	1 (2)	1 (2)	...	...	1 (2)	2	1
Ser	...	...	...	1 (...)	...	1 (...)	1	1	...	...	...
Thr	...	1	1	... (1)	1	... (1)	...	...	1	1	...
Tyr	...	...	...	...	...	...	1	1	...	...	...
Val	...	...	1	1	1	1	...	...	1	1	...
Seq. pos.[d]	189–194	151–154	250–265	250–265	250–265	250–265	155–171	155–171	250–265	250–265	6–16
Min. subs.[e]	0	0	1	2	1	2	1	1	1	0	1

[a] Designated peaks in profiles from Figures 1 and 2. Values in parentheses refer to differences in composition between the peptide and its equivalent in SMV-N coat protein (7). Equivalent peptides in SMV-N sequence could not be found for peaks A and B. The composition of peak C was not determined.

[b] Values rounded to the nearest integer. Trp and Cys not determined.

[c] Peak A was analyzed from strains G1, G3, G4, G5, G7, 75-16-1, 12-18, 0, Brazil; peak B from G1, G4, G6, G7; peak D from G5, VA (i), and Brazil (ii); peak E from 12-18, 0; peak F from Brazil; peak 7′ from G1; peak 17 from G3 (i) and 75-16-1 (ii); peak 17′ from G3, G5, G7; and peak 17″ from G1 and G4.

[d] Sequence position of the homologous peptides in the coat protein of SMV-N (Fig. 3).

[e] Minimum substitutions to account for the differences in the amino acid compositions as compared with the corresponding SMV-N peptides.

TABLE 2. Comparison of retention times of peaks from high-performance liquid chromatography profiles of potyvirus coat proteins from strains of soybean mosaic virus (SMV) and watermelon mosaic virus (WMV)

	Percentage of peaks with similar retention times[a,b]															
Potyvirus	SMV-N	SMV-G1	SMV-G2	SMV-G3	SMV-G4	SMV-G5	SMV-G6	SMV-G7	SMV-VA	SMV-Bra	SMV-Wis	SMV-12-18	SMV-75-16-1	SMV-0	WMV 2	PRSV-W
SMV-N	100	90	90	90	86	90	90	90	90	90	90	95	90	90	81	19
SMV-G1		100	100	100	95	100	100	95	95	95	95	90	90	90	86	19
SMV-G2			100	100	100	95	100	100	95	95	95	90	90	90	86	19
SMV-G3				100	100	95	100	100	95	95	95	95	90	90	86	19
SMV-G4					100	95	100	100	95	95	95	95	90	90	86	19
SMV-G5						100	95	95	100	100	100	95	95	95	80	20
SMV-G6							100	100	95	95	95	95	90	90	86	19
SMV-G7								100	95	95	95	95	90	90	86	19
SMV-VA									100	95	95	95	91	91	77	18
SMV-Bra										100	95	95	91	91	77	18
SMV-Wis											100	95	90	90	77	18
SMV-12-18												100	95	95	77	18
SMV-75-16-1													100	100	77	18
SMV-0														100	77	18
WMV 2															100	28
WMV 1																100

[a] The first sample-injection peaks and the last column-cleaning peak, common to all profiles, were omitted from comparisons.

[b] Peaks with retention times within 0.2 min of each other were considered to be similar.

potyviruses and their strains. As an extension of the above approach, we have assessed coat protein similarities in the potyvirus group by generating HPLC peptide profiles of coat protein tryptic digests. This technique requires only small quantities (50 μg) of coat protein, and initial data can be rapidly generated compared with the time required to determine complete coat protein sequences. Comparison of peak retention times in peptide profiles from coat proteins of distinct potyviruses and their strains revealed that a bimodal distribution of similarity of profiles exists similar to that for coat protein sequences (24). Pairwise comparisons of peptide profiles have shown that between 57 and 100% of peaks had identical retention times when strains of individual potyviruses were examined. In contrast, pairwise comparisons showed that between 16 and 42% of peaks from profiles of distinct potyviruses had the same retention times (23–25,32).

The results from HPLC peptide profiling of the 14 SMV strains suggest that all coat proteins have high sequence identity, since at least 90% of peaks have similar retention times (Figs. 1 and 2, Table 2).

Amino acid analysis of many of the HPLC peaks from all 14 SMV strains showed that many of the peptides were identical in composition to corresponding SMV-N peptides (Table 2). Those few peaks that had retention times different from those of SMV-N (peaks A–F, Figs. 1 and 2) were shown in most cases to have amino acid compositions that differed by only one or two amino acid residues from homologous SMV-N peptides (Table 1). These results provide detailed confirmation of the general conclusion, drawn from the peptide profile patterns, that the coat proteins of all 14 strains are very similar in sequence. Based on numerous previous observations (23–25,32,34–36), this conclusion indicates that the 14 SMV strains, including the SMV-VA strain investigated here, belong to the one potyvirus represented by SMV-N.

During the course of our work, Jayaram et al (19) reported cDNA sequences for the coat protein coding regions of a G2 and a G7 strain that revealed only two and three amino acid sequence differences, respectively, from the sequence of the coat protein of SMV-N. Our findings of very few sequence changes are in agreement with this data, although we found no evidence in our G7 coat protein for their reported (19) Met to Ile substitution at residue 217.

While WMV 2 is not as closely related to SMV-N as the other strains studied here, nearly 80% of the peak retention times on the WMV 2 coat protein profile were identical with those of SMV-N (Figs. 1 and 2, Table 2), confirming the high sequence similarity of the coat proteins of SMV-N (7) and WMV 2 (42). In contrast, only about 20% of peaks in the peptide profile of the distinct potyvirus PRSV-W (formerly WMV 1) have retention times identical with those of the SMV strains (Figs. 1 and 2, Table 2).

The fact that SMV-VA and SMV-N are strains of the same potyvirus on the basis of peptide profile information is very surprising. The reported sequence for SMV-VA (13) displayed a coat protein sequence identity of only 58% with SMV-N (7), and on this basis it was considered to be a distinct potyvirus (35). Coat protein peptide profiling with many potyviruses and their strains (23–25,32, N. McKern and D. D. Shukla, *unpublished results*) has consistently demonstrated that the peptide profiles of distinct potyviruses are very different. Thus, the peptide profiling data (Figs. 1 and 2) and amino acid compositions of individual peptides (Table 1) of SMV-VA do not conform to its reported coat protein sequence data (13), suggesting that the SMV-VA used here was not the same as that sequenced by Gunyuzlu et al (13). To confirm this conclusion, two preparations of SMV-VA, one purified in Blacksburg, VA, and the other in Ames, IA, were investigated. Coat proteins of both preparations gave essentially identical HPLC peptide profiles and amino acid compositions. Further examination revealed that the SMV-VA strain sequenced by Gunyuzlu et al (13) was derived from the original SMV-VA isolate (18) by a series of single local-lesion transfers. Thus, the original SMV-VA may have consisted of a mixture of two viruses, one closely related to SMV-N as the dominant virus and the other, the distinct potyvirus (SMV-VA) of Gunyuzlu et al (13), forming only a small part of the mixture. Support for this theory comes from the fact that SMV-VA has been shown, on the basis of its biological and serological properties (18), to be equivalent to the G1 strain of Cho and Goodman (5).

In conclusion, we have established that the SMV strains with different geographic origins (Table 3) belong to a single potyvirus, and they have coat protein amino acid sequences that are very similar to that of SMV-N (7). This is not unexpected because SMV is seedborne and soybean seeds are distributed worldwide, often harboring SMV. What is rather unexpected, however, is that SMV-VA had peptide profiles similar to SMV-N even though previous nucleotide sequence data demonstrated large differences (7,13). The reported sequence of the SMV-VA clone is clearly that of a potyvirus, and the most likely candidates for its identity include those potyviruses known to infect soybean (8). These include bean yellow mosaic virus (BYMV) (38); peanut stripe virus (PStV) (8); three Taiwanese isolates, PM, PN, and 74 (12), shown to be strains of blackeye cowpea mosaic/PStV/azuki bean mosaic viruses (23); peanut mottle virus (2); soybean crinkle virus (8); soybean yellow bud virus (8); and at least one other unidentified potyvirus isolated from soybeans in South Africa (26). The sequence data shows that it is not BYMV (14), PStV (22,23), or any of the other legume-infecting potyviruses for which sequence data are available (41). The culture did not react with antiserum to peanut mottle virus (39). In addition, the other candidate viruses are not known to be seedborne in soybean, and they were not cultured during the maintenance of the SMV-VA culture. We continue to study this discrepancy, and we hope to resolve it by reexamining the original culture and the cloned cDNA.

Finally, the relationship between WMV 2 and these SMV strains needs to be addressed. The question arises as to whether WMV 2 should be considered as a distinct virus that is closely related to SMV or as an SMV pathotype (9,10,32) in the same way that WMV 1 is accepted to be a pathotype of PRSV (27). Immunodiffusion tests have revealed a close serological relationship between WMV 2 and SMV similar to that between WMV 1 and PRSV, with spur formation occurring with one WMV 2 preparation but not the other (28). The coat protein sequence identities between WMV 1 and PRSV-P (total 97%, core 98%) are higher than those seen between SMV-N and WMV 2 (total 83%, core 92%), but the latter values are within the range between accepted strains of PVY, BYMV, PWV, PPV, JGMV, and SCMV (41). With regard to host range, the list of species that can be infected with known strains of SMV fall into only five plant families, with members of the *Leguminosae* (22 genera) predominating (6). WMV 2 also infects a large number of legumes (20 genera) but has a more extensive host range covering 22 plant families (6). However, the list of susceptible hosts for WMV 2 includes four of the five families, 17 of the 28 genera, and 22 of the 44 species (including soybean) that can be infected with SMV. This contrasts favorably with WMV 1 (now accepted as a strain of PRSV), which infects 38 species of *Cucurbitaceae* and two species of *Chenopodiaceae* but not papaya or other members of the *Caricaceae* family (27). PRSV-P, the type member, infects 15 species in the three plant families *Caricaceae, Chenopodiaceae,* and *Cucurbitaceae* (27). These points should be kept in mind when the classification of WMV 2 and SMV is considered.

TABLE 3. Origin of soybean mosaic virus strains examined

Isolate	Origin	Isolate	Origin
G1/019-2	Japan	75-16-1/G2	Iowa
G2/60	Liberia	12-18	Iowa
G3/83-2	Pakistan	0	Iowa
G4/016-2	Japan	VA/G1	Virginia
G5/32-2	Thailand	N/G2	Illinois
G6/U427	Korea	Wisconsin	Wisconsin
G7/U670	Korea	Brazil	Brazil

Initially, it was proposed (42) that SMV-N be considered a strain of WMV 2, since another distinct SMV potyvirus, SMV-VA (13), existed. Subsequently, it was suggested that more information on the viruses causing mosaic disease in soybeans was required before deciding whether SMV-N and WMV 2 were strains of SMV or WMV (10). The data presented here show that SMV-N is typical of the isolates infecting soybeans and should retain the name SMV. The appearance of new data and a reexamination of the past literature should establish whether WMV 2 is best classified as a pathotype of SMV or as a closely related but distinct virus.

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