Isolates of Bean Common Mosaic Virus Comprising Two Distinct Potyviruses

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ABSTRACT

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Strains of bean common mosaic virus (BCMV) have been identified largely by their characteristic interactions with a selected number of differential bean cultivars. Recent studies have suggested that BCMV strains could be classified on serological and other grounds as strains of two distinct potyviruses. Using high-performance liquid chromatographic peptide profiles of coat protein digests together with limited peptide analysis, 22 isolates of BCMV, including representatives from seven recognized pathotypes, were compared with each other and with the type strains of blackeye cowpea mosaic virus (BlCMV) and peanut stripe virus (PStV). The peptide profiles confirm the existence of two distinct potyviruses among the current isolates of BCMV. BCMV strains NL-3, NL-5, NL-8, and TN-1 are strains of one of the potyviruses, which we propose should be named "bean necrosis mosaic virus," since these strains induce temperature-insensitive necrosis in bean cultivars that carry the dominant I gene. Strains CH-2, NL-1, NL-2, NL-4, NL-6, NL-7, PR-1, RU-1, US-1, US-2 (three variant isolates), US-3, US-4, US-5, US-6, US-7, and US-10 are strains of the second potyvirus, which we suggest should retain the name BCMV. Peptide profile data also suggest that isolates of BlCMV, PStV, azuki bean mosaic virus (AzMV), and three potyvirus isolates from soybean (PM, PN, and 74) are all strains of the second potyvirus, BCMV.

Bean common mosaic virus (BCMV), a definitive member of the potyvirus group (28), is seed-transmitted in numerous *Phaseolus* cultivars. It has been reported from many parts of the world (3,20,24,35,50,54,56) and is perhaps the most common and the most destructive of the 34 viruses now known to naturally infect beans (3,24,35). Strains of the virus are identified on the basis of characteristic interactions with a selected number of bean cultivars and have been found to cause either common mosaic symptoms, which are sometimes associated with leaf malformation, or "black root," which is characterized by vascular necrosis and death of the plant (9). Serological and biological data have suggested that complex interrelationships exist among these strains and other potyviruses. Some recent reports suggest a close relationship between BCMV strains and other potyviruses that infect legumes. Lana et al (24) were unable to draw a clear line between some strains of BCMV, blackeye cowpea mosaic virus (BlCMV), and cowpea aphidborne mosaic virus (CAMV) following a detailed comparison of biological and serological properties. Tsuchizaki and Omura (50) concluded from similar studies that some isolates of BCMV, BlCMV, and azuki bean mosaic virus (AzMV) were strains of one potyvirus.

Although unambiguous taxonomic assignments have been difficult to achieve for many of the strains of distinct potyviruses now reported to naturally infect leguminous plant species using biological and serological criteria, those based on comparisons of coat proteins have been more successful. In most instances, gene sequences (including the 3′ noncoding region) or coat protein sequences can readily establish the virus or strain status of a particular isolate because of the significant difference in the sequence identities found between viruses and strains (41,42,57). Because of this distinction, simpler techniques such as nucleic acid hybridization, high-performance liquid chromatography (HPLC) peptide profiling, or N-terminal targeted serology can be used as cruder measures of genetic relatedness. For most virus isolates, such approaches are sufficient to decide if they are distinct viruses or related strains (57).

With regard to legume-infecting potyviruses, coat protein sequence data (57) has clearly distinguished strains of bean yellow mosaic virus from those of clover yellow vein virus (ClYVV). In addition, a comparison by HPLC of peptide profiles of coat proteins from 14 isolates of soybean mosaic virus has shown that all are strains of one potyvirus (19). Similarly, peptide profiles of AzMV, BlCMV, peanut stripe virus (PStV), and three isolates from soybean, indicated that all are strains of another potyvirus (30).

In a preliminary report (32) based on HPLC peptide profiles of two strains, we proposed that isolates of BCMV may consist of two or more distinct potyviruses. At the same time Vetten et al (54) have proposed that isolates of serogroups A and B of BCMV (34,56) represent two distinct viruses based on serological interactions and coat protein sequence data. In this paper we have investigated these proposals further, using peptide profiles and limited amino acid analyses of coat proteins from 22 isolates of BCMV that originated from Africa, Europe, and North and South America. The isolates studied span serogroups A and B, and they include representatives from seven known pathotypes (9,11). Type strains of BlCMV (25) and PStV (7) were also compared with the BCMV isolates. Our data provide substantial support for the proposal that BCMV strains can be divided into two distinct potyviruses, which correlate with the serogroups A

and B. The data further show that PStV and BlCMV are strains of the serogroup B potyvirus.

MATERIALS AND METHODS

Viral strains investigated. The potyvirus isolates investigated and their strain codes, identification, origin, and source are shown in Table 1. The isolates were purified according to method 2 of Reddick and Barnett (37).

Preparation of peptides and HPLC profiling. Enzyme digests were prepared by suspending 0.3–0.8 mg of freeze-dried viral preparations in 150–400 μl of 0.05 M ammonium bicarbonate by sonication, followed by incubation overnight at 37 C with trypsin (treated with *N*-tosyl-L-phenylalanine chloromethyl ketone) at a 1:50 enzyme-protein ratio. Solutions were dried, vortexed with 250–500 μl of 0.1% trifluoroacetic acid, and centrifuged at 9,000 *g* in a bench-top centrifuge. Soluble peptides were separated by injecting the solution onto a 5-μm Vydac reverse-phase C_{18} column (Separations Group, Hesperia, CA) connected to a liquid chromatograph (Series 4, Perkin-Elmer Corp., Norwalk, CT). Peptides were eluted from the column at 45 C by applying a linear gradient of 0–42% acetonitrile over 36 min at a flow rate of 2 ml/min and monitoring eluted peaks at 214 nm. All samples were chromatographed at least twice.

The 20 tallest peaks were compared, omitting those eluting within the first 4 min, which consisted of injection spikes, unbound peptides, and baseline noise at the commencement of the elution gradient. Retention times of the selected peaks were then compared on a pairwise basis with those from each of the other profiles (19). Pairs of peaks were considered to be the same if their retention times were within 0.2 min of each other.

Amino acid analysis of coat proteins and peptides. Prior to hydrolysis, some fractions were rechromatographed on the C_{18} column at 3 C in order to resolve peptide mixtures. Samples 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 and analyzed on a Water's Amino Acid Analyzer (Millipore Corporation, Bedford, MA) using an ion-exchange column.

RESULTS

Peptide profiles of coat proteins from BCMV strains NL-3, NL-5, NL-8, and TN-1 were very similar (Fig. 1). At least 90% of the 20 major peaks in these profiles had the same retention times (Table 2). In contrast, the peptide profile of NL-4 showed only 35–40% similarity to these four profiles (Table 2).

Figure 2 shows the peptide profiles obtained from tryptic digests of coat proteins from 18 strains of BCMV serogroup B, together with profiles from PStV-Stripe and BlCMV-Type. Amino acid analysis of peaks 1–20 from the NL-4 profile (Fig. 2) revealed that peptides within all but one of these were located within the known (54) coat protein sequence (Fig. 3). The residues identified in this manner accounted for 179 of the 286 sequence positions. They did not include the small N-terminal peptides commonly encountered in the column breakthrough peaks, or the insoluble 60-residue tryptic peptide from the coat protein core (Fig. 3).

Peptide profiles of BCMV isolates CH-2, NL-1, NL-2, NL-6, NL-7, PR-1, RU-1, US-1, US-2(D), US-2(P), US-2(Z), US-3, US-4, US-5, US-6, US-7, US-10, PStV-Stripe and BlCMV-Type were very similar to that of BCMV-NL4 (Fig. 2) and to each other. Between 60 and 95% of the 20 major peaks from each profile (mean 69 $\pm$ 7%) had coincident retention times (and generally similar relative peak heights) across the 210 pairwise comparisons of peptide profiles (Table 2). Differences between the various peptide profiles shown in Figure 2 were assessed by analyzing peaks a–j, which were present in only one or two of the profiles. Amino acid analysis of eight of these peaks (Table 3) indicated that they were composed of peptides of similar length (19–22 residues) and composition (varying by 2–6 amino acids). Since peak 12 of NL-4 was shown by amino acid analysis to correspond to the amino-terminal peptide of the NL-4 coat protein (54), these data suggest that peaks a–j contain the amino terminal peptides of the respective coat proteins. This conclusion has been directly confirmed (29) for the PStV coat protein (peak j in Fig. 2) and was supported for several of the other profiles by the finding that the peptides were blocked. This is commonly encountered for peptides originating from the N-terminus of potyvirus coat proteins (29).

TABLE 1. Identification, origin, and source of potyvirus isolates

Serogroup[a]	Pathogroup[b]	Strain code[c]	Strain identification	Origin	Original reference	Source[d]
A	III	NL-8	NL-8	Netherlands	11	A
	VI	NL-3	Michelite	Netherlands	15	A
		NL-5	Jolanda	Netherlands	15	A
		TN-1	Tanzania	Tanzania	44	A
B	I	US-1	Type	Washington	38	A
		NL-1	Westlandia	Netherlands	52	A
		PR-1	PR9M	Puerto Rico	1	A
	II	NL-7	NL-7	Peru	10	A
		US-7	R220	Washington	11	A
	IV	US-3	Idaho 123	Idaho	6	A
		US-4	Western	Washington	45	A
		US-5	Florida	Florida	58	A
		NL-6	Colana	Netherlands	16	A
		CH-2	Chile-2	Chile	55	A
	V	NL-2	RM	Netherlands	52	A
		US-2 (Z)	NY-15 (Z)	New York	38	A
		US-2 (D)	NY-15 (D)	Idaho	5	A
		US-2 (P)	NY-15 (P)	New York	23	A
	VII	NL-4	Great Northern	Netherlands	15	A
		US-6	Mexican	Washington	43	A
		US-10	NW-63	Washington	Unreported	A
		RU-1	Russian	USDA-PI	Unreported	A
		PStV	Stripe	China	7	Demski
		BlCMV	Type	Florida	25	Purcifull

[a] See Mink and Silbernagel (34); Vetten, Lesemann, and Maiss (54); and Wang, Mink, and Silbernagel (56).
[b] Drijfhout (9).
[c] See Drijfhout, Silbernagel, and Burke (11).
[d] A = Isolates were recovered from infected seed obtained from the original investigator wherever possible (see Drijfhout, Silbernagel, and Burke [11]) or isolated from infected plants grown at Prosser, Washington. The biological authenticity of each isolate was verified using the differential host range recommended in Drijfhout, Silbernagel, and Burke (11).

Small variations in retention times were observed between peaks common to many profiles in Figure 2. To clarify whether these variations were due to actual residue differences in component peptides or run-to-run variations, a number of peaks that had retention times similar to peak 10 in the NL-4 profile (Fig. 2) were analyzed. In NL-4 this peak contains the peptide Asn_{194}-Arg_{202} (Fig. 3). These peptides, with two exceptions, were found to have the same composition, indicating that small variations in retention time were due to run-to-run variation. In the case of BCMV-NL7 and BlCMV-Type, the peptide equivalent to peak 10 (marked by *) clearly elutes earlier than peak 10 of the other isolates. The NL-7 peak 10 equivalent contained a peptide with a Lys for Arg change in the 9-residue peptide Asn_{194}-Arg_{202} (Fig. 3), the same substitution previously observed (30) in the corresponding peptide (marked by *) from the BlCMV-Type profile.

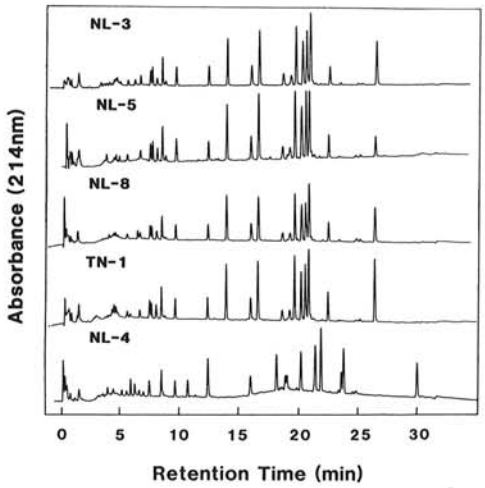

Fig. 1. Reverse-phase high-performance liquid chromatography of tryptic digests of coat proteins from five strains of BCMV. The origin, source, and identification of these strains are shown in Table 1. Peptides bound to the column were eluted with a linear gradient of 0–42% acetonitrile in 0.1% aqueous trifluoroacetic acid over 36 min at a flow rate of 2 ml/min and column temperature of 45 C.

Several peptides equivalent to peak 20 of NL-4 were also analyzed. Each had an amino acid composition which corresponded exactly to the 24-residue, peak 20 peptide of NL-4 (Gly_{80}-Arg_{103}; Fig. 3), except for the peptides from isolates NL-1 and US-5, both of which had a Ser for Asp (or Asn) substitution. In the case of US-5, an accompanying reduction in the retention time of peak 20 was also observed.

Amino acid analyses revealed that the differences observed in retention times of some of the peaks in the set that eluted around 19–22 min (Fig. 2) could be ascribed to sequence changes in the C-terminal peptides of the coat proteins. The single peak 16 of NL-4 contained two coeluting peptides, Gln_{177}-Arg_{193} and the C-terminal peptide Asp_{272}-Gln_{287} (Fig. 3). These peptides were partially resolved in the profiles of US-1, US-3, NL-7, US-7, PR-1, NL-1, CH-2, US-5, and US-6 (Fig. 2). Amino acid analysis of one of these (US-1) revealed no differences between the partially resolved peak in US-1 and the unresolved peak 16 of NL-4. The two peptides were completely resolved into two separate peaks in the profiles of BlCMV-Type and the BCMV strains NL-2, RU-1, US-2 (variants D, P, and Z), and US-4 (Fig. 2). Compositional analysis of the NL-2 peaks showed that this separation was due to an amino acid substitution (Ser for Pro) within the C-terminal peptide Asp_{272}-Gln_{287} (Fig. 3), causing a reduction in the retention time of this peptide and resulting in

TABLE 2. Percentage similarity of peptide profiles of 22 isolates of bean common mosaic virus (BCMV), the Stripe strain of peanut stripe virus (PStV), and the Type strain of blackeye cowpea mosaic virus (BlCMV)[a]

	Potyvirus strain[b]																						
BCMV	NL-3	NL-5	NL-8	TN-1	CH-2	NL-1	NL-2	NL-4	NL-6	NL-7	PR-1	RU-1	US-1	US-2 (D)	US-2 (P)	US-2 (Z)	US-3	US-4	US-5	US-6	US-7	US-10	PStV
NL-5	95																						
NL-8	95	90																					
TN-1	90	95	90																				
CH-2	35	35	35	40																			
NL-1	35	35	25	30	75																		
NL-2	35	45	30	40	65	65																	
NL-4	35	40	35	40	65	75	65																
NL-6	40	40	35	35	70	60	65	70															
NL-7	30	35	40	40	75	75	60	80	65														
PR-1	40	45	45	35	70	85	65	75	60	75													
RU-1	35	45	45	40	65	75	70	70	65	60	70												
US-1	35	30	40	40	75	85	70	80	65	80	85	75											
US-2(D)	40	40	45	40	70	70	70	65	65	65	70	95	65										
US-2(P)	50	40	35	40	75	65	70	70	60	60	70	65	70	80									
US-2(Z)	40	45	45	40	65	60	70	60	65	60	65	80	60	85	65								
US-3	45	40	35	45	70	80	60	80	70	75	75	85	75	60	65	60							
US-4	45	40	40	45	65	75	70	70	70	70	75	80	65	80	75	80	70						
US-5	40	40	45	45	65	70	60	75	60	65	75	60	65	70	60	65	65	65					
US-6	45	35	35	45	65	75	60	80	60	70	70	75	70	65	70	70	85	65	80				
US-7	40	35	35	40	70	65	65	75	60	70	80	75	95	65	65	60	75	60	60	65			
US-10	30	45	45	35	65	60	60	65	60	65	65	65	70	65	70	65	65	60	65	75	75		
PStV	35	35	45	35	60	65	65	65	60	65	60	75	70	70	65	65	65	75	65	65	60	65	
BlCMV	40	40	40	40	65	65	65	70	60	80	60	75	70	70	60	65	75	75	60	70	60	65	60
BCMV	NL-3	NL-5	NL-8	TN-1	CH-2	NL-1	NL-2	NL-4	NL-6	NL-7	PR-1	RU-1	US-1	US-2 (D)	US-2 (P)	US-2 (Z)	US-3	US-4	US-5	US-6	US-7	US-10	PStV

[a] Tryptic digests of coat proteins of 22 potyvirus isolates were digested with trypsin and soluble peptides analyzed by reverse-phase high-performance liquid chromatography. Peptide profiles as shown in Figures 1 and 2 were obtained and assessed by comparing retention times of the 20 tallest peaks from each of a pair of profiles. Values of 60% or greater are boxed.

[b] See Table 1 for strain codes of the potyvirus isolates.

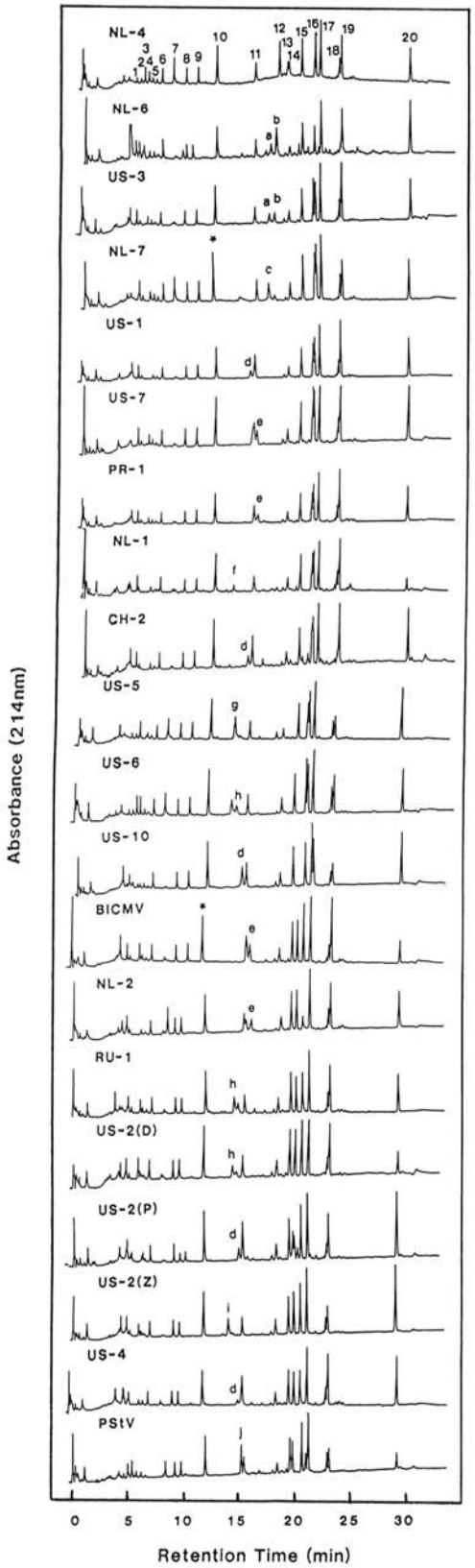

Fig. 2. Reverse-phase high-performance liquid chromatography of tryptic digests of coat proteins from nineteen strains of bean common mosaic virus (BCMV), together with those from the Stripe strain of peanut stripe virus and the Type strain of blackeye cowpea mosaic virus. The origin, source, and identification of these isolates are shown in Table 1. Peptides bound to the column were eluted using conditions identical to those described for Figure 1. Numbered peaks of BCMV-NL4, together with peaks a–j and those with retention time similar to peaks 10 and 20, were collected and analyzed.

the appearance of four, rather than three, peaks in this region of the profile. In BlCMV-Type and BlCMV-W this Ser for Pro substitution has been shown (30) to occur at Pro_{285}, not Pro_{286}. The similar pattern observed in the profiles BlCMV-Type, RU-1, US-2 (variants D, P, and Z), and US-4 (Fig.2), was presumably due to the same single residue change observed for NL-2. The further reduction in the retention time of the C-terminal peptide in the PStV profile is due to a second substitution of Ala for Pro at position 286 (Fig. 3).

DISCUSSION

It is now established that coat protein sequence data can be used to determine whether isolates of potyviruses are distinct potyviruses or strains of the one virus (40,41,42,57). Studies have shown that HPLC peptide profiles provide reliable data with which to compare coat protein sequences (19,21,30,31,35,39). Peptide profiles of coat proteins from strains of a single potyvirus are very similar, with at least half of the major peaks having coincident retention times. In contrast, in those cases examined to date, peptide profiles derived from distinct potyviruses shared only a minority of peaks with common retention times. Peptide profiles of coat proteins therefore provide a means for rapid examination of the relationships among potyvirus isolates.

The results of the present study show that the BCMV strains examined here belong to two distinct viruses. The first virus is composed of the strains NL-3, NL-5, NL-8 and TN-1, whose coat protein peptide profiles are very nearly superimposable, indicating that the coat proteins are very similar. This is in agreement with recent data that shows that the coat proteins of BCMV-NL3, BCMV-NL5 (P. Mills, *personal communication*), and BCMV-NL8 (54) have greater than 95% sequence identity, confirming that they are all strains of the same potyvirus. The second virus comprises the other 18 strains of BCMV examined here, as well as BlCMV and PStV (Fig. 2). The peptide profiles of these are quite distinct from those of NL-3, NL-5, NL-8, and TN-1 (average profile similarity 39 ± 5%; Table 2) but are closely related to each other (average profile similarity 69 ± 7%; Table 2), which is within the range previously observed among strains of potyviruses (19,21,30,31,35,39). The only substantial differences observed were in peptides originating from the N-termini of these isolates (Table 3).

Serological, biological, and biochemical data support the division of BCMV isolates into two distinct viruses. First, this assignment exactly matches the serological delineation of BCMV strains into serogroups A and B (34,56). A similar result has recently been obtained by Vetten et al (54), using an overlapping set of BCMV isolates. Although previous serological studies using double-antibody sandwich enzyme-linked immunosorbent assay

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                          20                  40                  60
          <-------T12--------->                <--T7--><---T2-->
BCMV-NL4  SGSGHPPLPVVDAGVDTEKDKKDKSSRGKDPENKEETRNNSRGTENPTMRDKDVNAGSRG
PStV        TSTQS  LN     A    E  NK  G  SS GSG        QS          K

                          80                  100                 120
          <T6>       <-T14->                <--T18/19->
BCMV-NL4  KVVPRLQRITKRMNLPMVKGNVILNLDHLLDYKPEQTDLFNTRATKMQFEMWYNAVKGEY
PStV       I     K

                          140                 160                 180
                                                                  <---
BCMV-NL4  EIDDDQMAIIMNGFMVWCIDNGTSPDVNGTWVMMDGDEQVEYPLKPMVENAKPTLRQIMH
PStV          E  S V

                          200                 220                 240
          ----T16----->                <--T11>     <T5><---T17--->      <--T8->
BCMV-NL4  HFSDAAEAYIEMRNSERPYMPRYGLLRNLRDKNLARYAFDFYEVTSKTSDRAREAVAQMK
PStV

                          260                 280
          <---T9--->                <------T16----->
BCMV-NL4  AAALSNVSSKLFGLDGNVATTSENTERHTARDVNQNMHTLLGMGPPQ
PStV            N                                   SA
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Fig. 3. Location of tryptic peptides in the sequence of BCMV-NL4 coat protein (54). The elution position (e.g., T12) of the numbered peaks containing these peptides is shown in Figure 2. The PStV-Stripe coat protein sequence (29,30) is shown for comparison. A gap was introduced at position 11 in the BCMV-NL4 sequence to maximize alignment with the PStV-Stripe sequence.

(24) suggested that there was a distant relationship between BCMV-NL3 on the one hand and BCMV-NL1 and NY-15 on the other, this was shown (20) to be due to antibodies directed to the core region of the coat proteins, which have high sequence identity throughout most of the potyvirus group (40,41,42,57). When these core antibodies were removed by cross absorption (20), the remaining antibodies, directed to the N-terminus (41,42) of BCMV-NL3, were virus-specific and did not cross-react with NL-1 or NY-15. This indicated that BCMV-NL3 was distinct from the other two BCMV strains.

Second, this assignment correlates well with that based on biological properties (Table 1). For example, only the members of serogroup A cause the temperature-insensitive necrosis reaction in bean cultivars possessing the dominant I gene (11,34). Finally, complete coat protein sequence data from BCMV-NL8 (serogroup A) and BCMV-NL4 (serogroup B) confirms the distinct potyvirus status of these two strains, since their coat proteins showed only 78% identity, close to the range of 35–75% observed for distinct species (40,41,42,57) and well below the 90% or more observed for strains.

Previous peptide profiles (30) indicated that AzMV, three potyvirus isolates from soybean (13), and other PStV and BlCMV strains had coat protein sequences that were closely related to PStV-Stripe and BlCMV-Type. A comparison of that data with the results presented here shows that those viruses (30) are also very similar to the serogroup B strains of BCMV. Furthermore, the recently published coat protein sequence of PStV (29) confirms its close relationship with the serogroup B strains of BCMV, since it has approximately 90% identity with that of BCMV-NL4 (54). This is within the range observed for strains of the one potyvirus (40,41,42,57). Thus there are many potyvirus isolates, including AzMV, BlCMV, PStV, and the serogroup B strains of BCMV, that are members of the second potyvirus.

A reexamination of previous literature supports these conclusions. Tsuchizaki and Omura (50) concluded that two BCMV isolates, two BlCMV isolates, and AzMV were strains of one potyvirus, since 1) they were serologically identical on agar gel diffusion; 2) their coat proteins had similar M_r on SDS polyacrylamide gel electrophoresis; 3) *Staphylococcus* V8 protease digestion of their coat proteins gave nearly identical cleavage patterns when examined by SDS gel electrophoresis; and 4) positive cross-protection was found between BCMV and BlCMV, between BCMV and AzMV, and between BlCMV and AzMV. With regard to host range, BCMV has been reported to infect 100 species of 44 genera of nine plant families, including four of the five families, 13 of the 16 genera, and 23 of the 34 species reported to be infected by BlCMV (12). Both BCMV and BlCMV infect cowpea and azuki bean. In the description of BlCMV (36), the fact that BCMV is not listed among the viruses that cause similar symptoms in cowpea suggests that, when found in cowpea, BCMV is typed as BlCMV. In addition AzMV, BlCMV, and BCMV have been reported to induce the same (subdivision I) cylindrical inclusion (CI) morphology (12).

The situation with PStV and the soybean isolates PM, PN, and 74 is less clear. Green et al (13) showed that the soybean isolates could infect French bean, cowpea, and azuki bean and reported a close serological relationship between these isolates and AzMV, BlCMV, BCMV, and the mosaic strain of PStV. However, the CI morphology and host ranges for the soybean isolates and PStV differ from those of AzMV, BCMV, and BlCMV (53). This CI inclusion morphology is subdivision IV rather than subdivision I, and neither BCMV nor BlCMV has been reported to be able to infect peanut, although BCMV can infect all other species and genera reported as hosts for PStV (12). The soybean isolates are intermediate in host range, since soybean PN was capable of infecting two of the nine peanut cultivars that were susceptible to PStV, but at a much lower frequency (53).

These differences in host range should not preclude the inclusion of PStV and the soybean isolates as strains of the same virus that includes AzMV, BlCMV, and the serogroup B strains of BCMV, since the situation is similar to that found with the two pathotypes of papaya ringspot virus (PRSV). The two strains

TABLE 3. Amino acid compositions of the N-terminal tryptic peptides from isolates of bean common mosaic virus (BCMV), peanut stripe virus (PStV), and blackeye cowpea mosaic virus (BlCMV)[a]

	Residues per molecule[c]							
	Peak[d]							
Amino acid[b]	12	b	c	d	e	g	h	j
Ala	1	...	1	1	2	1	1	2
Arg	...	...	...	...	1	...	...	...
Asx	3	2	3	1	3	4	3	2
Glx	1	3	1	3	2	...	...	1
Gly	3	4	3	4	4	3	4	2
His	1	...	1	...	...	1	1	...
Ile	...	2	1	1	1	...	...	...
Leu	1	...	...	...	...	...	...	1
Lys	2	2	2	1	2	2	2	1
Met	...	...	...	...	...	...	...	...
Phe	...	...	...	...	...	...	...	...
Pro	3	3	4	3	3	4	4	1
Ser	2	2	1	2	1	2	2	4
Thr	1	1	1	1	1	1	1	3
Tyr	...	...	...	...	...	...	...	...
Val	3	2	3	2	2	3	3	2
Sequence position	1–21	1–21	1–21	1–19	1–22	1–21	1–21	1–19
Differences with −NL4[e]		5	2	4	5	2	2	6

[a] See Materials and Methods for conditions for generation of peptides and acid hyrolysis.
[b] Cys and Trp not determined.
[c] Values rounded to the nearest integer.
[d] Numbers and letters refer to peaks designated in Figure 2. Analyzed peptides were from the following profiles: 12 = BCMV-NL4; b = BCMV-NL3; c = BCMV-NL7; d = BCMV-US1; e = BlCMV Type; g = BCMV-US5; h = BCMV-US6; j = PStV Stripe. Peptides corresponding to peaks a, f, and i were not analyzed.
[e] Minimum number of residue differences between the sample and the N-terminal peptide in peak 12 of BCMV-NL4 that were consistent with the differences in amino acid composition.

PRSV-P and PRSV-W (formerly watermelon mosaic virus 1) show high coat protein sequence identity (57), and both infect various members of the Cucurbitaceae and Chenopodiaceae families. However, only PRSV-P can infect papaya and other members of the Caricaceae family (12). We recommend that these pathotype differences be regarded as strain discriminators rather than species discriminators, since it is known that host range can be expanded by successive passage through intermediate host plants. A major argument for the creation of PStV as a new potyvirus (7) was its capacity to infect peanuts, whereas other legume-infecting potyviruses, such as BlCMV, could not. No serological differences were observed between PStV and BlCMV (7). From their comparison of the soybean isolates PN, 74, and PM with PStV, Vetten et al (53) concluded that the soybean isolates were strains of PStV that were adapted to soybean, their principal host plant. From the data presented here, this proposal is extended to suggest that the soybean isolates and PStV are strains of the serogroup B BCMV potyvirus that are adapted to soybean and peanut, respectively.

With regard to nomenclature, it is proposed that the potyvirus that includes AzMV, BlCMV, PStV, the serogroup B strains of BCMV, and the three isolates from soybean should retain the name *bean common mosaic virus*. This nomenclature appears appropriate for two reasons. First, the name BCMV takes precedence, as it was described (47) prior to AzMV (27), BlCMV (2), and PStV (7). Second, the acronym BCMV is appropriate because the large number and widespread distribution of the serogroup B isolates suggests that this potyvirus is more common than the serogroup A virus. A recent examination of the U.S. Department of Agriculture *Phaseolus* germ plasm collection revealed that BCMV contamination was a serious problem, particularly in *P. vulgaris* accessions, where approximately 60% were infected. All of these BCMV infections were typed as serogroup B (22). In addition, the inclusion of strains of BlCMV, PStV, AzMV, and the three soybean isolates as members of this potyvirus makes it even more common, since BlCMV and PStV are already widespread pathogens (33).

These BCMV strains appear to be quite distinct from the temperature-insensitive, necrosis-inducing serogroup A strains which, on the basis of several properties, could be renamed *bean necrotic mosaic virus*, as suggested by Vetten et al (54), *bean necrosis virus*, or *bean necrosis mosaic virus*. The name *bean necrosis virus* was used to describe a bean disease in Oregon (14), which is now known to be caused by ClYVV (R. O. Hampton, *personal communication*). We favor the name *bean necrosis mosaic virus* (BNMV), since it characterizes the two alternate symptoms induced by the virus in different bean cultivars, and since there is a precedent for the use of such combined descriptors in the name *rice necrosis mosaic virus* (18). The acronym BNMV is not listed in the recently proposed set of acronyms for plant viruses and viroids (17). This proposed new classification will require new nomenclature, as discussed previously (42).

The relationship between CAMV (26) and these two distinct potyviruses remains to be established. AzMV has been listed as a suspected synonym of BCMV (46), BlCMV (4), and CAMV (28) and has been shown to protect azuki bean plants from infection by a Japanese isolate of CAMV (51). Dijkstra et al (8), from a study of host range, symptoms, and serology, concluded that BlCMV-Fla, BlCMV-NR, and CAMV-Morocco were strains of the one virus, although CAMV-Morocco was serologically distinct from the BlCMV strains. In contrast, Taiwo et al (48,49) concluded that CAMV-Kenya and CAMV-Nigeria were strains of BlCMV, and CAMV-Morocco and CAMV-Cyprus were not strains of BlCMV, but were strains of a distinct potyvirus. This raises the possibility that some isolates of CAMV might also be members of the widespread group of BCMV strains.

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