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# Identification and classification of potyviruses on the basis of coat protein sequence data and serology

## Brief Review

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**Summary.** The identification and classification of potyviruses has been in a very unsatisfactory state due to the large size of the group, the apparent vast variation among the members and the lack satisfactory taxonomic parameters that will distinguish distinct viruses from strains. In the past, use of classical methods, such as host range and symptomatology, cross-protection, morphology of cytoplasmic inclusions and conventional serology, revealed a "continuum" implying that the "species" and "strain" concepts cannot be applied to potyvirses. In contrast nucleic acid and amino acid sequence data of coat proteins has clearly demonstrated that potyviruses can be divided into distinct members and strains. This sequence data in combination with information of the structure of the potyvirus particle has been used to develop simple techniques such as HPLC peptide profiling, serology (using polyclonal antibody probes obtained by cross-adsorption with core protein from trypsin treated particles) and cDNA hybridization. These findings, along with immunochemical analyses of overlapping synthetic peptides have established the molecular basis for potyvirus serology; explained many of the problems associated with the application of conventional serology; and provided a sound basis for the identification and classification of potyviruses. As a result, the virus/strain status of some potyviruses has been redefined, requiring a change in the potyvirus nomenclature. These new developments necessiate a re-evaluation of the earlier literature on symptomatology, cross-protection, cytoplasmic inclusion body morphology and serology.

## Introduction

The potyvirus group is the largest and economically most important of the 28 plant virus groups and families currently recognized [67]. The potyvirus group was established in 1959 as one of the plant virus groups with elongated particles

[12]. At that time the number of viruses recognized in the group was only 14. Since then a large number of new members have been discovered and added to the list of potyviruses to make it the most rapidly growing of all the plant virus groups [30]. By 1971 the number of potyviruses had increased to 25 [106], by 1976 to 45 [28], by 1979 to 73 [65], by 1982 to 115 [67], by 1985 to 152 [30], and by 1988 to 175 [49, 54, 59, 60, 70, 82, 92, 96, 99].

Although it has been suggested that many viruses included in the group may be synonymous [29], recent molecular analysis reveals that the number of distinct viruses being recognized is increasing much faster than the number of recognized synonyms. Thus, while pepper mottle virus (PeMV) appears to be a strain of potato virus Y (PVY) [86] and the N strain of soybean mosaic virus (SMV-N) a strain of watermelon mosaic virus 2 (WMV 2) [31, 109], sugarcane mosaic virus (SCMV) and SMV have each been shown to consist of four distinct potyviruses [82, 92, unpubl. results] and bean yellow mosaic virus (BYMV) strains to consist of more than one distinct virus [41, 96]. Thus, it appears that the number of distinct potyviruses recognized is going to increase dramatically. While the present 175 definitive and possible members account for about 30% of all known plant viruses, we believe that this figure may increase to 50% once the strains of many of the potyviruses are properly characterized, particularly those infecting legumes [7, 10, 16, 51, 58, 98].

Most potyviruses have narrow, often extremely restricted host ranges. They flourish in a wide range of crops and environmental conditions [46, 47]. In 1974 they were reported to infect 1,112 species of 369 genera in 53 plant families [23]. Since then many more species, genera and families would have been added as hosts of potyviruses. Their economic importance is highlighted by the fact that, in a recent survey of the ten most important filamentous viruses from each of the ten major world regions, 73% were potyviruses [70].

Definitive potyviruses are transmitted in the non-persistent manner by many aphid species while some possible members have fungus, mite or whitefly vectors. Definitive and possible members of the group investigated so far have all been found to induce characteristic "pinwheel" cytoplasmic inclusion bodies in infected plant cells [46, 47].

Potyvirus particles are flexuous rods, 680–900 nm long and 11 nm wide, consisting of a single protein species of $M_r$ ranging from 30,000 to 37,000 and a single molecular of ssRNA of $M_r$ $3.0 \times 10^6$ to $3.5 \times 10^6$ [46]. For a detailed description of the structure of potyvirus particles and their coat proteins, see Shukla and Ward [83] and for genome organization and function, see Dougherty and Carrington [19].

It has been repeatedly pointed out by taxonomists and reviewers that the taxonomy of the potyvirus group is in a very unsatisfactory state and that successful resolution of potyvirus taxonomy presents a major challenge for plant virologists [30, 42, 46, 47] that would have tested Linnaeus [70]. The current unsatisfactory state of potyvirus taxonomy has been due to the large size of the group, the apparent vast variation among the members and the lack

of satisfactory taxonomic parameters that will distinguish distinct viruses from strains [29, 30, 70]. It has been suggested, on the basis of comparative biological properties and inconsistent serology, that strains of potyviruses form a "continuous" array between two or more distinct viruses in such a way that the borderlines separating individual potyviruses cannot be sharply defined [10, 42, 46, 47, 58]. In this way a distant strain of one virus could appear more closely related to a distant strain of a second virus than either are to their homologous viruses [42, 46, 47]. However, recent findings on potyvirus particle structure [89], serology [91–93], and coat protein sequences [82, 83] cast doubt on the "continuum" hypothesis.

The aims of the present paper are to briefly review the classical approaches used in the past to differentiate between distinct viruses and strains, to highlight the difficulties posed by the use of such methods and to discuss the recent developments in our understanding of potyvirus coat protein structure and particle assembly and its implication for the serology and taxonomy of potyviruses.

## Classical approaches to identification and classification

### *Symptomatology and host range*

Symptomatology and host range have played a significant role in the delineation of potyvirues and their strains in the past and even today they remain the first criteria for recognition of strains. Since most potyviruses have restricted host ranges, they can often be identified on the basis of the characteristic symptoms they produce in certain hosts. However, reliance on these criteria has created a lot of confusion in the identification of potyviruses infecting members of some plant families, such as *Leguminosae* [7, 10, 16, 51, 58, 98] and *Gramineae* [92]. Here different potyviruses have been shown to produce similar symptoms in some hosts while different climatic conditions, different cultivars or different genetic lines of the same plant species can have profound effects on the susceptibility to and symptoms of potyvirus infections [10, 46, 47]. For example, the four distinct potyviruses now recognized to infect maize, sorghum, and sugarcane produce similar symptoms in a number of maize cultivars and sorghum lines [92]. Similarly, different potyviruses causing mosaic disease of soybean in the United States cannot be easily identified on the basis of their reactions on soybean cultivars because the symptoms produced are very similar [unpubl. results]. Furthermore, Bos and co-workers [10, 16, 58] observed that the different potyviruses infecting legumes had many hosts in common and differed only slightly in the range of effects on the host plants. Bos [10] also found that his clover yellow vein (CYVV) and cowpea aphid-borne mosaic (CAMV) viruses produced systemic symptoms in pea, cv. Koroza, in contrast to the reports that the former did not infect peas [48] and the later caused only latent infection in this plant species [62]. These few examples highlight the problem of using host range and symptomatology for identification and clas-

sification of potyviruses. Some of these problems may be resolved by standardization of hosts, varieties and climatic conditions [11]. Host range and symptomatology can play a vital role in the identification and classification of potyviruses if the biochemical properties of the viruses are established first and then efforts are made to find differential hosts which can distinguish one virus from the other similar viruses. This approach was recently used successfully to differentiate four potyviruses infecting *Gramineae* [92] on the basis of their reactions on selected sorghum inbreds [81; M. Tosic, pers. comm.].

### *Cross-protection*

It is generally accepted that related strains of the same virus are capable of cross-protection, whereas distinct viruses are not, and this property has been used successfully in the past for identification of plant viruses. In the beginning, cross-protection tests were given considerable weight in establishing whether virus isolates were related strains or not. However, doubt about the value of cross-protection data arose when correlations with other taxonomic parameters diverged [66].

Examples where complete protection was shown between distinct strains of the one potyvirus are BYMV [10, 38], SCMV [97] [61], and WMV2 [50]. Examples of unexpected cross-protection between viruses hitherto considered to be distinct are BYMV and SMV [74], BYMV and bean common mosaic virus (BCMV) [74], SMV and BCMV [74], BYMV and red clover necrosis virus [110], pea mosaic virus and BYMV [35], lettuce mosaic virus (LMV) and CAMV [10]. Examples of unexpected failed cross-protection are the lack of effect of mild strains of ryegrass mosaic virus on infection of rye plants with necrotic strains [107] and the failure of strain A of maize dwarf mosaic virus (MDMV) to prevent multiplication of the B strain of MDMV in sorghum [73]. Such observations indicated the need for caution when interpreting cross-protection results [66].

Some of the conflicting results in cross-protection experiments with potyviruses may be due to technical problems. Most potyviruses cause mosaic symptoms, and cross-protection is better achieved if the challenging strain causes necrotic local lesions [66]. However, in our opinion a large number of the conflicting results may be attributed to misidentification of the viruses and strains used. For example, some strains of SCMV are known to cross-protect each other while others (such a strains A and B of MDMV) do not [73, 80, 97]. When it was found that the strains of SCMV consist of four distinct potyviruses, not one [92], the previous cross-protection results with these strains neatly conformed to their assignments as four distinct viruses [92]. Since SMV and BYMV are also heterogeneous mixtures of more than one virus [41, 82, 96] much of the published information on unexpected cross-protection involving these viruses needs re-examination. Thus, cross-protection may prove to be very useful as a taxonomic criterion for potyviruses once the assignment of the viruses and strains compared to date has been corrected.

### *Cytoplasmic inclusions*

Possible and definitive members of the potyvirus group that have been examined so far have all been found to induce the characteristic "pinwheel" type inclusions in infected cells [23], and this property has been considered the single most important criterion for assigning viruses to the potyvirus group [91]. These inclusions are formed by assembly of the cytoplasmic inclusion protein, one of the products obtained by post-translation cleavage of the large polyprotein translated from the potyviral genome [6, 17, 64]. On the basis of morphology of these inclusions, Edwardson and co-workers [23, 24] divided potyviruses into four subgroups. Viruses in the subgroup I produce tubular and scroll-like inclusions; those in the subgroup II are characterized by laminated aggregates; those in subgroup III produce scrolls and laminated aggregates; while viruses of subgroup IV produce scrolls and short, curved laminated aggregates. The morphology of cytoplasmic inclusions should reflect the primary structure of the inclusion protein and thus help in the identification of some potyviruses. It could also find use in establishing an hierarchical classification of the potyvirus group. Francki et al. [30] believe the latter to be hazardous since different strains of the same virus fall into different subgroups, distinct viruses have exactly the same inclusion morphology and distinct viruses, which are serologically related, belong to different subgroups. However, given the need to revise the virus/strain status of many potyviruses [31, 41, 81–83, 86, 92, 96, 109] and the finding that many serological cross-reactions do not reflect genetic relatedness [89, 90, 93], the whole question of the value of cytoplasmic inclusions as taxonomic markers remains an open one. We believe that proper identification of viruses and strains will resolve most of the problems associated with the classification of potyviruses based on morphology of the cytoplasmic inclusions. For example, strains A, D, and E of MDMV exhibit subgroup I morphology [23] whereas the B strain of MDMV and the A and E strains of SCMV belong the subgroup III [22, 23] despite the fact that all these strains were believed to be strains of SCMV. However, recent findings [92] have shown that the SCMV strains consist of four distinct viruses, MDMV, SCMV, Johnsongrass mosaic virus (JGMV), and sorghum mosaic virus with MDMV-A, MDMV-D, and MDMV-E classified as strains of MDMV and SCMV-A, SCMV-E, and MDMV-B as strains of SCMV. The cytoplasmic inclusion subgroupings for these viruses are in perfect agreement with this classification.

## Identification and classification based on molecular structure

In an excellent review of the historical developments of general taxonomic principles, Mayr [68] drew attention to Darwin's comment that taxonomy reflects propinquity of descent and that all true classifications are genealogical. Mayr [68] also traced the changes in criteria used for general taxonomy from the use of descriptive morphological characters to the application of biochemical techniques that characterize the variation and evolution of molecules. Plant

virus taxonomy has gone through similar phases of development from the initial reliance on morphological and biological properties to assignments based on coat protein and nucleic acid sequences.

### *Nucleic acid sequences and hybridization*

Nucleic acid hybridization is a potentially useful approach for establishing the identity of a new isolate, and this technique has been used successfully for the detection and identification of several potyviruses [1, 2, 3, 7–9, 77, 104]. Nucleotide sequences of the genomes of three potyviruses [6, 17, 64] reveal that some parts of the genome are more homologous than the others. However, in no case was the nucleic acid sequence homology extensive. A computer comparison of nucleotide sequences of the entire genome of tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV) [6, 17] revealed the presence of only seven matches of sequences of 15 or more (15 to 21) nucleotides, the minimum oligonucleotide length required for positive cDNA hybridization. In contrast, a search for identical peptide regions, seven or more amino acid residues long (enough to form an epitope), revealed the presence of 41 matching regions (7–18 residues long) in the polyprotein ($M_r$ about 340,000) encoded by the genomes of these two viruses. Thus, nucleic acid hybridization does not appear to be useful for establishing hierarchical relationships among potyviruses [2, 3, 7, 77].

Nucleic acid sequence data and nucleic acid hybridization can, however, be used to identify potyviruses and to discriminate viruses from strains. Frenkel et al. [31] observed that the nucleotide sequence of the 3′-untranslated region of the potyvirus RNA can serve as a basis for identification and classification of potyviruses. This region in distinct potyviruses differs considerably in length and displays no significant sequence homology. In contrast, in related strains of the same virus the 3′-untranslated region is very similar in length and sequence. cDNA probes involving the 3′-untranslated region of the potyviral RNA should, therefore, be able to detect all strains of a potyvirus.

### *Coat protein structure*

Criteria based on the structural properties of coat proteins may be more useful and more easily applied than other properties for identification and classification of potyviruses for the following reasons [82, 83]. Firstly, the coat protein is a unique gene product with an amino acid composition that is characteristic of the group [26, 27] and an amino acid sequence that shows no significant homology between different groups of plant viruses, in contrast to other gene products [18]. Secondly, the coat protein is the only major gene product in the virion and accounts for 95% of the potyvirus particles. It has been argued in the past that the coat protein represents only a fraction of total coding capacity of the viral genome (approximately 12% in the case of potyviruses) and therefore, taxonomic criteria based on coat protein will compare only a fraction of

the total genomic information. However, as has been pointed out by Van Regenmortel [102], successful classifications have been developed on the basis of very few parameters and the reconstruction of phylogenetic trees of higher organisms has been done on the basis of the degree of sequence homology found in a small number of their proteins [108]. Finally, serology reflects protein structure and serological techniques are the most preferred to date among the various methods used for detection, identification and classification of plant viruses.

## Amino acid composition

Analysis of all available data on the amino acid composition of the coat proteins of members in different plant virus groups has convincingly demonstrated that the amino acid composition of coat proteins is characteristic of the virus group [26, 27]. With potyviruses, coat protein amino acid composition has been used successfully, though only on a limited scale, as a taxonomic criterion for differentiating the individual viruses within the group [36, 71, 75]. However, as pointed out be Milne [70], this approach may be more useful for distinguishing potyviruses from other virus groups.

## Amino acid sequence homology

We have recently shown that coat protein amino acid sequence data can be used to identify and differentiate distinct potyviruses and their strains [82, 83]. Complete coat protein amino acid sequences are now known for 25 strains of 11 distinct potyviruses [4, 5, 17, 20, 25, 31, 37, 40, 41, 57, 63, 64, 76, 84–87, 96, 100, 109; J. Jilka and J. M. Clark Jr., pers. comm.]. As shown in Fig. 1 the coat proteins from distinct potyviruses vary considerably in size (263–330 amino acids) because of differences in the length of their N-termini. The N-terminal regions in distinct viruses also vary in sequence whereas the C-terminal three quarters of the coat proteins are highly homologous (65% identity). In contrast, the related strains of the same virus (e.g., Fig. 2) generally have coat proteins of the same length and their N-terminal sequences are highly homologous.

Mild proteolysis by trypsin of intact particles of distinct potyviruses has revealed that the trypsin-susceptible, N- and C-terminal regions of their coat proteins are exposed on the surface of the virus particles. The core particles (devoid of N- and C-termini, as indicated by arrows in Fig. 1) cannot be distinguished from the untreated particles under the electron microscope and are still infectious [89].

Analysis of the 136 possible pairings of the complete coat protein amino acid sequences from 17 strains of eight distinct potyviruses (Fig. 3) revealed a bimodal distribution of sequence homology [82]. In this analysis the sequence homology between distinct members ranged from 38 to 71% (average 54%) while that between strains of the one virus ranged from 90 to 99% (average

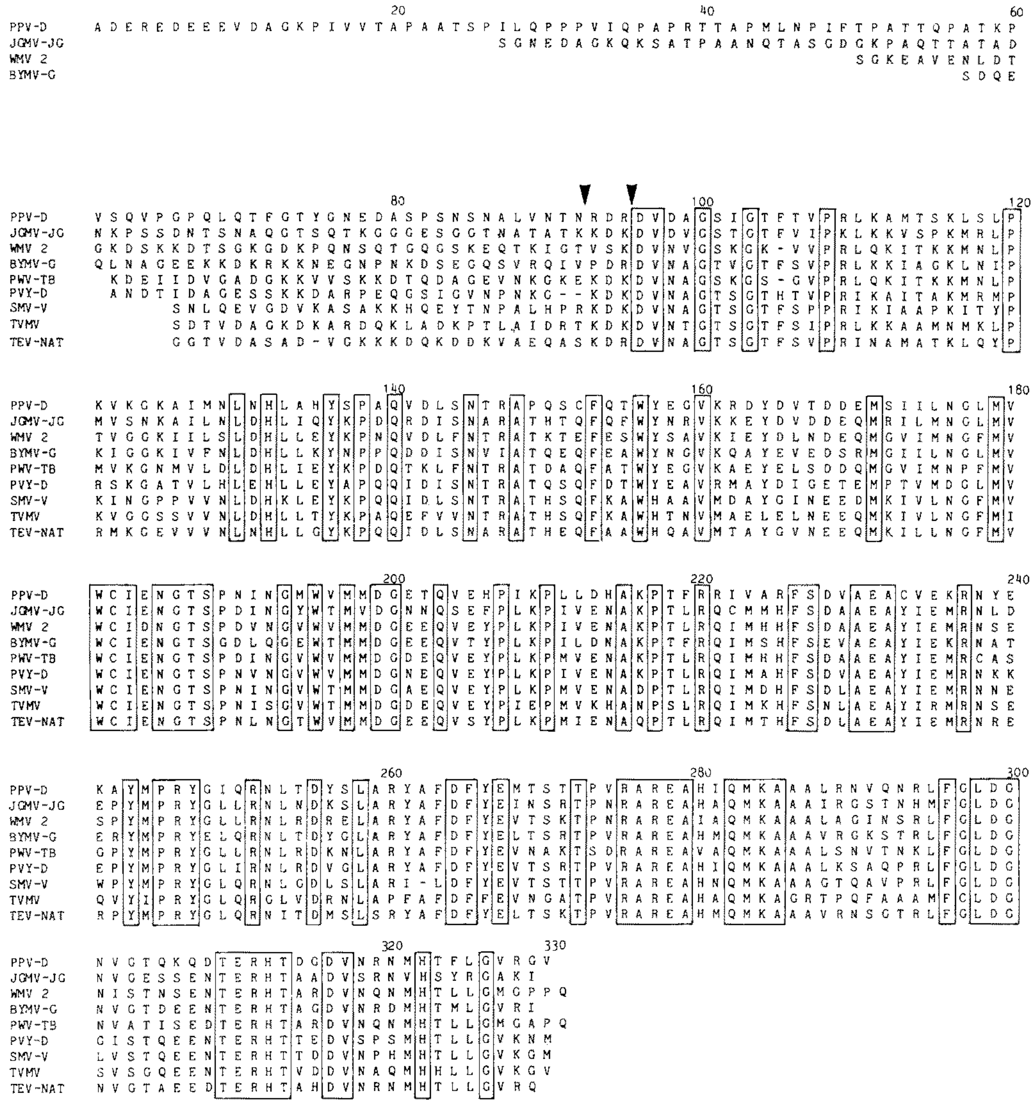


**Fig. 1.** A comparison of amino acid sequences of coat proteins from nine potyviruses. The sources of the sequence data are listed in Table 1. The boxed amino acid residues are identical among the nine proteins. ▶ Location of the new N- and C-termini of the core protein produced after mild treatment with trypsin

95%). These findings are not consistent with the "continuum" hypothesis proposed to explain the unsatisfactory taxonomy of potyviruses and show a clear demarcation of sequence homology between distinct potyviruses and strains. The sequence homology between distinct members was little affected by the choice of strain used to make the comparison, indicating that, at least for the viruses examined, the boundaries between the peripheral virus strains were not blurred [82].

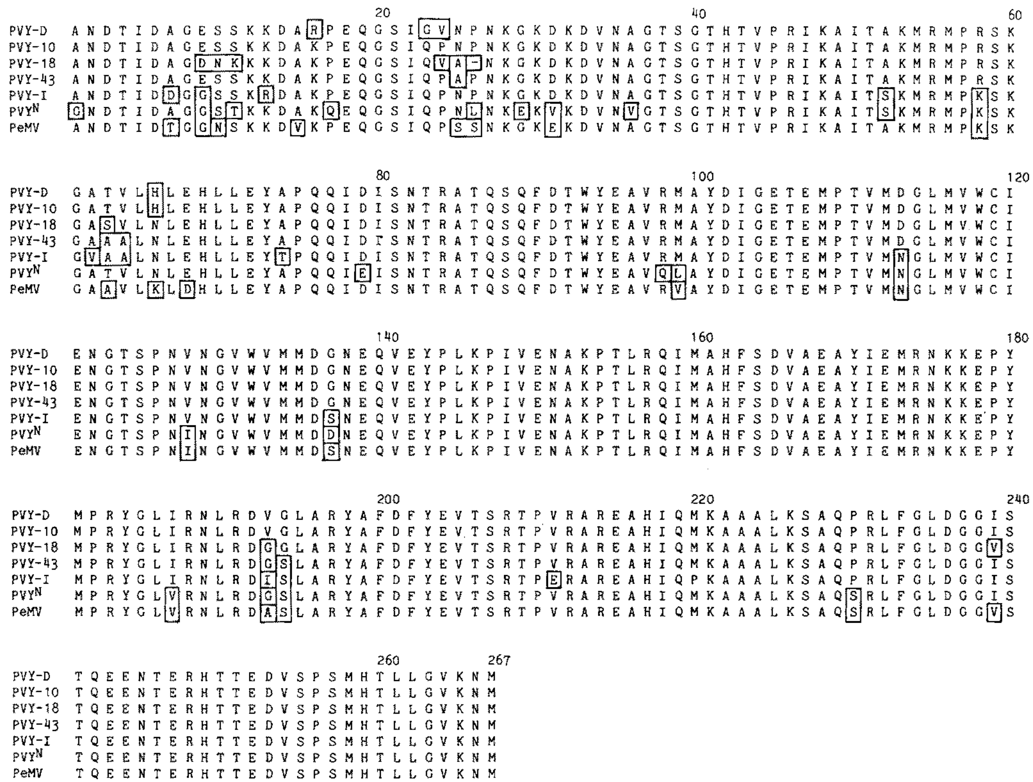

**Fig. 2.** A comparison of amino acid sequences of coat proteins of seven strains of PVY. The sources of the sequence data are listed in Table 2. The boxed amino acid residues represent variations in the seven proteins

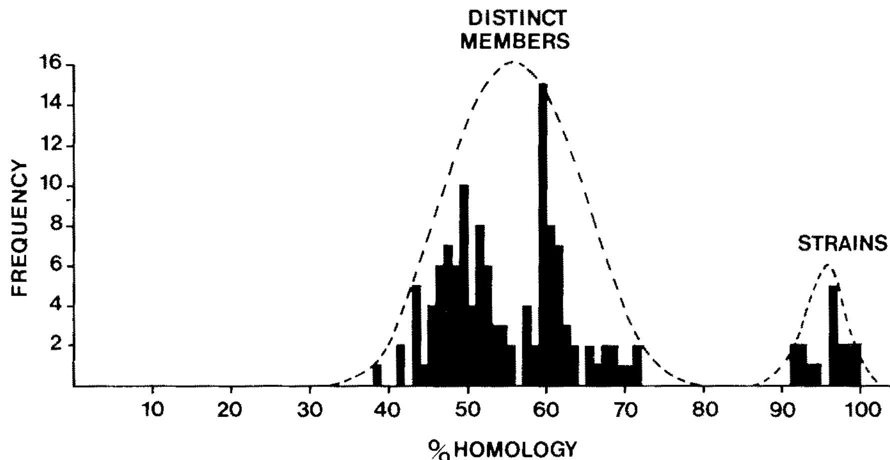


**Fig. 3.** Frequency distribution of the amino acid sequence homologies for the coat proteins from 17 strains of eight distinct potyviruses. Reproduced from Shukla and Ward [82]

**Table 1.** Percent amino acid sequence homology between coat proteins of potyvirus strains[a]

| | BYMV | | PPV | | | | PVY | | | | | | | PWV | | | TEV | | WMV 2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | CS | D | AT | NAT | R | D | 10 | 18 | 43 | I | N | PeMV | TB | S | M | NAT | HAT | WMV 2 | SMV-N |
| BYMV-G | – | 88.3 | | | | | | | | | | | | | | | | | | |
| BYMV-CS | | – | | | | | | | | | | | | | | | | | | |
| PPV-D | | | – | 95.5 | 90.6 | 94.8 | | | | | | | | | | | | | | |
| PPV-AT | | | | – | 94.2 | 98.8 | | | | | | | | | | | | | | |
| PPV-NAT | | | | | – | 93.6 | | | | | | | | | | | | | | |
| PPV-R | | | | | | – | | | | | | | | | | | | | | |
| PVY-D | | | | | | | – | 89.9 | 95.9 | 96.6 | 93.3 | 91.0 | 91.4 | | | | | | | |
| PVY-10 | | | | | | | | – | 96.3 | 97.8 | 94.4 | 92.1 | 92.5 | | | | | | | |
| PVY-18 | | | | | | | | | – | 96.6 | 92.9 | 91.0 | 92.1 | | | | | | | |
| PVY-43 | | | | | | | | | | – | 95.5 | 92.1 | 92.9 | | | | | | | |
| PVY-I | | | | | | | | | | | – | 91.8 | 92.9 | | | | | | | |
| PVY$^N$ | | | | | | | | | | | | – | 92.1 | | | | | | | |
| PeMV | | | | | | | | | | | | | – | | | | | | | |
| PWV-TB | | | | | | | | | | | | | | – | 98.9 | 95.9 | | | | |
| PWV-S | | | | | | | | | | | | | | | – | 95.5 | | | | |
| PWV-M | | | | | | | | | | | | | | | | – | | | | |
| TEV-NAT | | | | | | | | | | | | | | | | | – | 97.7 | | |
| TEV-HAT | | | | | | | | | | | | | | | | | | – | | |
| WMV 2 | | | | | | | | | | | | | | | | | | | – | 82.6 |
| SMV-N | | | | | | | | | | | | | | | | | | | | – |

[a] The sources of sequence data were: BYMV-G [41], BYMV-CS [96], PPV-D [76], PPV-AT and PPV-NAT [63, 64], PPV-R [57]. PVY-D [84], PVY-10, PVY-18, and PVY-43 [86], PVY-I [78], PVY$^N$ [100], PeMV [20], PWV-TB, PWV-S, and PWV-M [87]. TEV-NAT and TEV-HAT [4, 5], WMV 2 [31, 109], SMV-N [25]

**Table 2.** Percent amino acid sequence homology between coat proteins of distinct potyviruses[a]

| | BYMV-G | BYMV-30 | JGMV-JG | PPV-D | PVY-D | PWV-TB | MDMV-B | SMV-V | TEV-NAT | TVMV | WMV 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BYMV-G | – | 69.6 | 50.5 | 47.9 | 67.3 | 63.8 | 47.0 | 56.0 | 60.4 | 49.6 | 55.5 |
| BYMV-30 | | – | 49.5 | 43.9 | 49.6 | 51.3 | 43.6 | 49.3 | 54.2 | 44.5 | 52.1 |
| JGMV-JG | | | – | 46.7 | 49.0 | 51.5 | 55.2 | 46.7 | 48.2 | 45.7 | 54.1 |
| PPV-D | | | | – | 47.3 | 43.2 | 47.8 | 43.0 | 47.6 | 40.3 | 43.9 |
| PVY-D | | | | | – | 60.0 | 46.2 | 65.5 | 61.8 | 53.6 | 60.0 |
| PWV-TB | | | | | | – | 43.9 | 55.2 | 51.1 | 52.6 | 68.7 |
| SCMV-MDMV-B | | | | | | | – | 42.4 | 48.5 | 39.9 | 47.0 |
| SMV-V | | | | | | | | – | 68.3 | 64.5 | 54.3 |
| TEV-NAT | | | | | | | | | – | 57.4 | 56.4 |
| TVMV | | | | | | | | | | – | 50.5 |
| WMV 2 | | | | | | | | | | | – |

[a] The sources of sequence data were: BYMV-G [41], BYMV-CS [96], JGMV-JG [85], PPV-D [76], PVY-D [84], PWV-TB [87], SCMV-MDMV-B [J. Jilka and J. M. Clark Jr., pers. comm.], SMV-V [40], TEV-NAT [5], TVMV [17], WMV 2 [31, 109]. Sequence identities were calculated as described previously [82], but with a break penalty of 10 to allow alignment of homologous N-terminal sections with gaps [83]

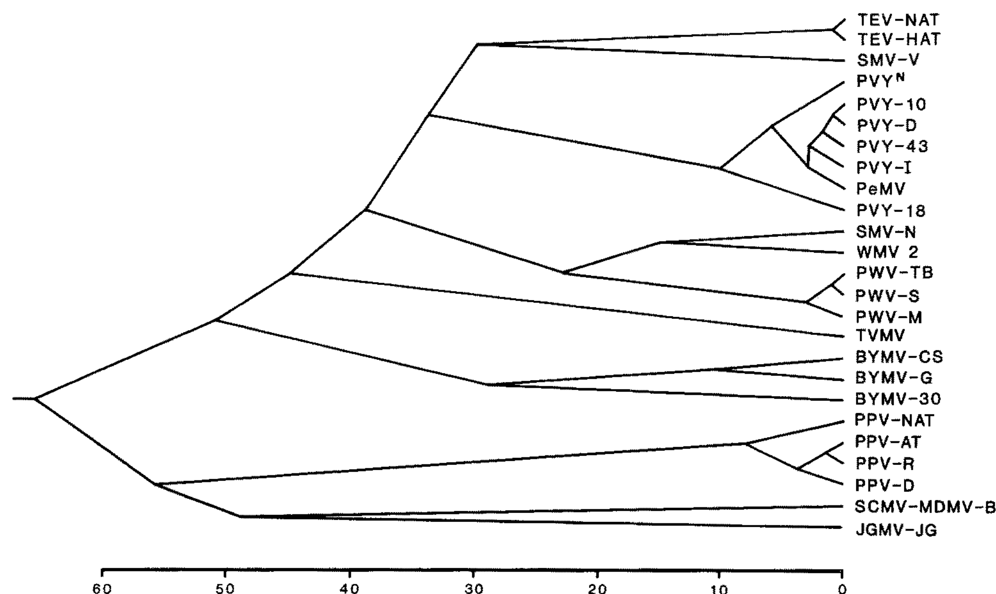


**Fig. 4.** A dendrogram calculated from the coat protein sequence similarities of 25 strains of eleven distinct potyviruses. First the progressive alignment method of Feng and Doolittle [27A] was used to establish the distances for all comparisons, then these were used to calculate a dendrogram by the UPMGA method of Sneath and Sokal [95]. Evolutionary difference is a measure of the acutal change that occurred during evolution. The sources of the sequence data are listed in Tables 1 and 2. [A. J. Gibbs et al., unpubl. results]

Since that report [82], coat protein sequences of the R strain of plum pox virus (PPV) [57], the I and N strains of PVY [78, 100], three strains of BYMV, BYMV-G [41], BYMV CS, and BYMV-30 [96], WMV 2 [31, 109], and the MDMV-B strain [J. Jilka and J. M. Clark Jr., pers. comm.] of SCMV [92] have become available. Comparison of the new sequences confirmed the strain status of PPV-R and PVY-I and $PVY^N$ as they have more than 90% sequence identity with other strains of their respective viruses (Table 1). Similarly, the sequence of MDMV-B strain of SCMV showed more than 90% sequence identity when compared with the available partial sequence data for three SCMV strains [85]. In contrast, only two of the BYMV isolates (G and CS) show high sequence identity (88%) and appear to be true strains of BYMV. The third isolate BYMV-30 showed a much lower level of sequence identity (70–73%) suggesting that it is a distinct member of the potyvirus group (Tables 1 and 2). Comparison of the coat protein sequences of WMV 2 with other potyviruses showed sequence homology ranging from 44 to 69% (Table 2) except with the N strain of SMV [25] where the sequence identity with WMV 2 was 83% [109]. At first glance this degree of sequence homology appears to be almost midway between that found for distinct potyviruses and that for related strains. However, close examination of the two sequences revealed that the lower se-

quence identity results from a 16 residue deletion in the N-terminal region of SMV-N. If this deletion is treated as a single mutational event, the sequence homology between SMV-N and WMV2 rises to 90%. Moreover, the degree of homology and the location of sequence differences between WMV2 and SMV-N is much closer to that observed between strains of the same virus than that found between distinct potyviruses. Furthermore, the high homology in the 3′-untranslated region of genome of the two viruses also supports our suggestion that WMV2 and SMV-N are strains of the same virus [31].

It is interesting to note that the bimodal distribution of sequence homologies obtained so far with potyviruses is similar to that found for the coat proteins of subtypes (39–70%) and strains (83–99%) of influenza virus [82].

Although amino acid sequence homology between coat proteins of potyviruses clearly differentiates between distinct members and strains, its value as a taxonomic criterion would be greatly enhanced if this property could be exploited by simpler techniques than sequencing. High-performance liquid chromatographic (HPLC) peptide profiling of coat protein digests [88] and serology based on defined epitopes [89, 90, 92, 93] described in the following sections suggest that such an approach is possible. Finally it would be interesting to see whether coat protein sequences can be used to establish hierarchical relationships between distinct members of the potyvirus group. As a first attempt a dendrogram showing the structural relationships of all available amino acid sequence data from coat proteins of potyviruses and their strains is presented in Fig. 4 [A. J. Gibbs et al., unpubl. results].

## High performance liquid chromatographic peptide profiling

Using tryptic digests of coat proteins from four strains of PVY, three strains of SCMV, two strains of passionfruit woodiness virus (PWV), and one strain each of BYMV, JGMV, and WMV2, Shukla et al. [88] showed that HPLC peptide profiling had the potential to aid the identification and classification of potyviruses. The authors showed that the peptide patterns of strains from the same virus were very similar but those from distinct potyviruses were quite different (Fig. 5). The method is based on the different degrees of coat protein sequence homology found between distinct members of the potyvirus group on the one hand and between strains of the one potyvirus on the other and compares the entire coat proteins of potyvirues [88]. Depending on the availability of facilities, exact differences between proteins can be determined by this method as most of the peaks in Fig. 5 represent single peptides which can be directly sequenced. Using this approach it was recently shown that 13 potyvirus strains causing mosaic disease of soybean in the United States and Brazil and currently classified as strains of SMV belong in fact to four distinct potyviruses [D. D. Shukla et al., unpubl. results].

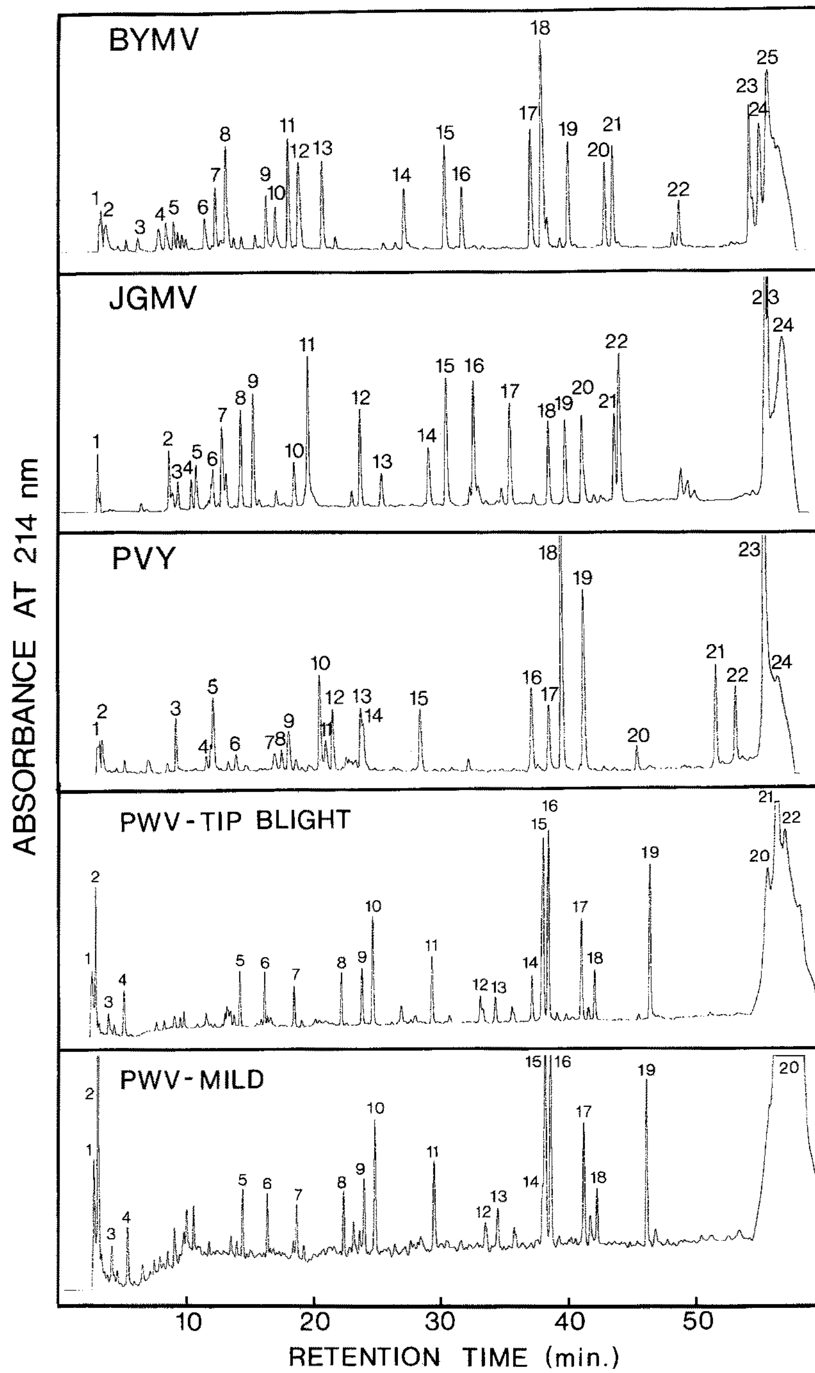


**Fig. 5.** High performance liquid chromatographic peptide profiles of tryptic digests of coat proteins of four distinct potyviruses (BYMV, JGMV, PVY, PWV) and two strains of PWV. Adapted from Shukla et al. [88]

## Identification and classification based on serology

### *Classical serology*

Among the various properties used in the past for the identification and classification of potyviruses, serological tests appear to offer the most practical method for establishing the identity of a new isolate [46, 47, 71, 72], and therefore, serology has been used extensively for the detection and establishment of relationships among potyviruses [101]. However, serological relationships among distinct members of the potyvirus group are extremely complex. It has been suggested that there is no simple pattern of antigenic relationships among members in the group and it has been observed that serological relationships often do not correlate with biological properties [42, 46, 47]. In this respect potyviruses are perhaps exceptions to the rule that viruses which are antigenically related also share most of their other properties [29]. Hollings and Brunt [46] noted that although most definitive members are serologically related to at least one other member in the group and in many cases to several others, the expected serological relationships between many connected pairs have not been observed. For example, BYMV has been found to be related to LMV and BCMV but no serological relationship between LMV and BCMV has been observed. Most puzzling are the genuine serological relationships reported between viruses infecting monocots and dicots such as the strong serological relationship between JGMV and WMV2 [89] and that between the HAT strain of TEV and the MDMV-B strain of SCMV [90]. JGMV and SCMV are known to have hosts only in the monocot family *Gramineae* whereas WMV2 and TEV primarily infect members in the dicot families *Cucurbitaceae* and *Solanaceae*, respectively. It has also been observed that antisera prepared against a potyvirus in one laboratory may differ considerably in their specificities from antisera to the same virus raised in another laboratory [14], thus confusing the serological relationships between the viruses involved. It is also known that antisera to dissociated coat proteins have much broader specificities than those produced to intact virus particles [79, 89], and that antisera produced to coat proteins prepared by different procedures can differ in their specificities [43]. On the basis of such observations it has been suggested that unless considerable caution is used in the interpretation of serological data, serology may be a misleading approach for tracing relationships among potyviruses [29, 71].

Various explanations have been proposed to account for the complex nature of the serological relationships among potyviruses [30, 55]. Use of antisera of widely different titres, different assay techniques and conditions, use of only one or few antisera instead of many bleedings from the same animal and partial proteolytic in situ degradation of the coat proteins are some of the factors suggested to be responsible for the contradictory results in the serology of potyviruses [30, 55]. However, we believe that the complexity in serology of potyviruses can only be resolved by a thorough understanding of the variation in coat protein structure within the potyvirus group [82, 83, 89, 90, 92, 93].

*New approaches with polyclonal antisera*

Recent findings from structural and immunochemical studies of potyvirus coat proteins from our laboratory [89, 90, 92, 93] have established the molecular basis for serology of potyviruses and have indicated how such information can be used to rationally design simple serological techniques for the accurate identification and classification of potyviruses. The results have also provided explanations for much of the past confusion in the serology of potyviruses.

Our structural and immunochemical studies revealed that the N- and C-termini of the coat proteins are surface-located and that the N-terminus constitutes the most immunodominant region in the potyvirus particles [89]. Since the surface-exposed N-terminus is the only large region in the entire potyvirus coat protein that is variable and virus-specific, epitopes contained in this region should generate virus-specific antibodies. On the other hand, the core protein region in different potyviruses shows considerable sequence identity and antibodies to this region should be excellent broad spectrum probes capable of detecting most, if not all, potyviruses [89]. On the basis of the above information, Shukla et al. [90] have developed a simple affinity chromatographic procedure to obtain virus-specific antibodies from polyclonal antisera raised against intact particles of potyviruses. The method involved: (1) removal of the virus-specific N-terminal region of the coat protein from particles of one potyvirus using lysyl endopeptidase, (2) coupling the truncated coat protein to cyanogen bromide-activated Sepharose, and (3) passing antisera to different potyviruses through the column. Antibodies that did not bind to the column were found to be directed to the N-terminus of the coat proteins and were highly specific (Fig. 6). Such an approach was employed recently to show that 17 potyvirus strains infecting *Gramineae* were not all closely related strains of SCMV, as previously believed, but represented four distinct potyviruses (Table 3), namely JGMV, MDMV, SCMV, and sorghum mosaic virus [92]. Our results also demonstrated that antibodies raised against the dissociated coat protein core (devoid of N- and C-termini) of a potyvirus (JGMV) recognized (Fig. 7A) all 15 different definitive aphid-transmitted as well as mite- and whitefly-transmitted potyviruses [89–91]. These results clearly demonstrated that virus-specific and group-specific monoclonal or polyclonal antibody probes to potyviruses can be produced by targeting the immune response to either the virus-specific, N-terminal region (29 to 95 amino acid residues depending on the virus) or the conserved core region (216 amino acids) of the coat proteins, respectively (Fig. 1).

Much of the contradictory information on serological relationships among potviruses can be attributed to the presence in antisera of variable proportions of cross-reacting antibodies that are targeted to the coat protein core of potyviruses. Substantial variation in specificity was observed when 11 potyvirus antisera produced in different laboratories were tested with 12 distinct potyviruses [90]. A majority of the antisera recognized all or most of the potyviruses

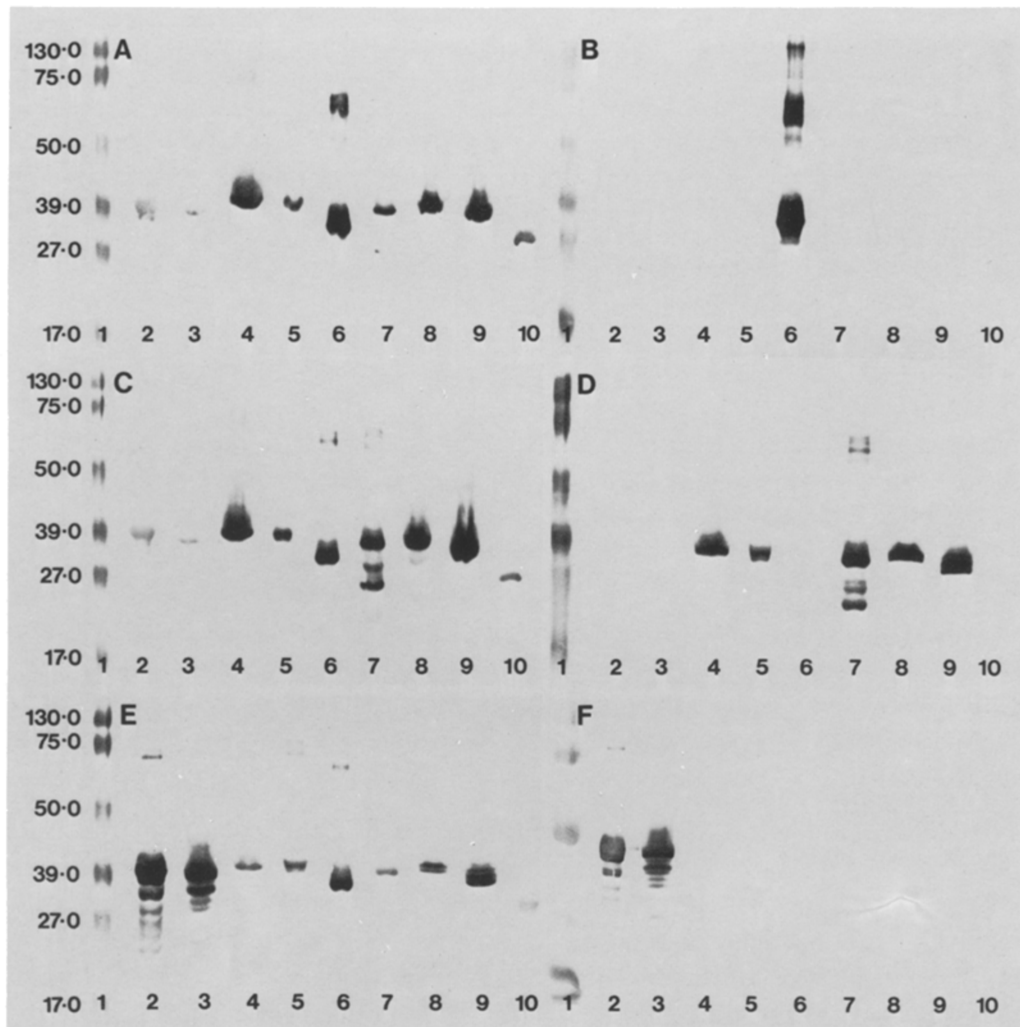


**Fig 6.** Electro-blot immunoassay of strains of JGMV, MDMV, and SCMV. *1* Bio-Rad (**A–E**) or BRL prestained standards (**F**). *2–10* JGMV-JG, JGMV-MDMV-O, SCMV-SC, SCMV-E, MDMV-A, SCMV-MDMV-B, SCMV-BC, SCMV-Sabi, and TEV, respectively. **A, C,** and **E** Probed with unfractionated antisera to MDMV-A, SCMV-MDMV-B, and JGMV-MDMV-O, respectively. **B, D,** and **F** Probed with affinity-purified virus-specific antisera directed to N-terminus of coat proteins of MDMV-A, SCMV-MDMV-B, and JGMV-MDMV-O, respectively. Reproduced from Shukla et al. [92]

tested whereas two antisera reacted only with their homologous viruses. Such variation in the specificity of the antisera may be due to either of two factors. Firstly, the state of the purified virus preparations used for immunization may have contributed to this situation. It is known that the N- and C-termini of coat proteins of potyviruses are degraded during purification and storage by enzymes of plant or microbial origin which co-sediment with the virus particle [89]. The degradation is faster with some viruses than with others. Some viruses

**Table 3.** Grouping of maize dwarf mosaic virus and sugarcane mosaic virus strains from Australia and the United States on the basis of reactivities of affinity purified virus-specific antibodies

| JGMV[a] | MDMV[b] | SCMV[c] | SrMV[d] |
|---|---|---|---|
| SCMV-JG (Aust.)[e] | MDMV-A (U.S.) | MDMV-B (U.S.) | SCMV-H (U.S.) |
| MDMV-O (U.S.)[f] | MDMV-D (U.S.) | SCMV-A (U.S.) | SCMV-I (U.S.) |
| | MDMV-E (U.S.) | SCMV-B (U.S.) | SCMV-M (U.S.) |
| | MDMV-F (U.S.) | SCMV-D (U.S.) | |
| | | SCMV-E (U.S.) | |
| | | SCMV-SC (Aust.) | |
| | | SCMV-BC (Aust.) | |
| | | SCMV-Sabi (Aust.) | |

Reproduced from Shukla et al. [92]
[a] JGMV Johnsongrass mosaic virus
[b] MDMV Maize dwarf mosaic virus
[c] SCMV Sugarcane mosaic virus
[d] SrMV Sorghum mosaic virus
[e] Aust. Australia
[f] U.S. United States

may completely lose their termini even after storage for a short period [90]. The usual practice in different laboratories is to use the same preparation of purified virus for successive immunizations. Since the N-terminus contains the virus-specific epitopes, its removal from virus particles in situ would gradually result in virus particles containing only non-specific core epitopes. Secondly, immunization procedures may have had an influence. There is considerable variation in the literature on the number, interval and route of injections, and the amount of antigen administered when producing an antiserum to plant viruses [101]. Although there is little reliable information available concerning the relative merits of different immunization procedures, these are very likely to affect the reactivities of the antibodies produced. Large differences in the reactivity of antisera taken at different stages of immunization of the same animal have been reported [56]; antisera from early bleedings contain virus-specific antibodies whereas cross-reacting antibodies begin to appear in later stages of immunizations [103]. Our investigation of potyviruses gave similar results. First and second bleedings (obtained after two and five injections) from a rabbit immunized with intact JGMV particles contained antibodies directed to the N-terminal region only whereas the final bleeding (obtained after a total of seven injections) contained antibodies also to the core region [89, 93]. Similarly, the first bleeding from a rabbit (obtained after 4 weeks of immunization) immunized with MDMV-A was found to be specific for the virus whereas three later bleedings obtained after 8, 11, and 13 weeks reacted with biologically and serologically distinct potyviruses [92].

*New approaches with monoclonal antibodies*

Monoclonal antibodies have now been produced to several potyviruses [13, 15, 21, 39, 44, 45, 52, 53, 69, 93; D. R. Hewish et al., unpubl. results]. Results from these studies reveal that immunization of mice with intact potyvirus particles generate monoclonal antibodies of different specificities. Some recognize only their homologous viruses and their strains while others react with 2, 3, 4, and in some cases with most or all potyviruses (Fig. 7B) [53, 93]. Since cloning and selecting lines with desired reactivities is often a laborious procedure and constitutes the major limitation to the rapid establishment of lines producing antibodies useful for diagnostic purposes [45], we have developed a simple method to identify virus-specific antibodies. The method involves screening

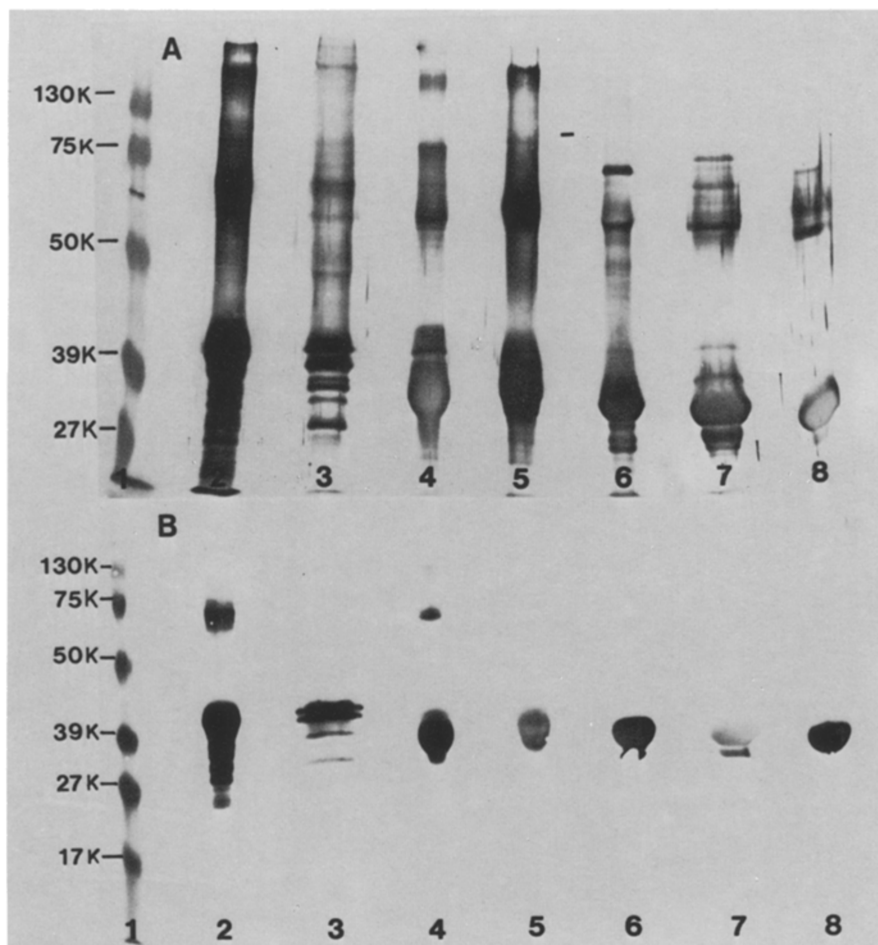


**Fig. 7.** Electro-blot immunoassay of six distinct viruses and two strains of the one virus. *1* Bio-Rad prestained standard, *2–8* JGMV-JG, JGMV-MDMV-O, BYMV, PWV, PVY, SMV-V, and TEV, respectively. **A** Probed with denatured JGMV core protein antiserum, **B** probed with monoclonal antibody 1/16 raised to intact particles of JGMV. Adapted from Shukla et al. [93]

against native and truncated (minus N-terminus) coat proteins in electro-blot immunoassay. Monoclonals directed to the N-terminus will only recognize the native protein band, whereas those directed to coat protein core will react with native as well as core protein bands [D. R. Hewish et al., unpubl. results]. We have shown that the epitopes contained in the surface exposed N-terminal region of potyviruses are sequential, and therefore will react in electro-blot immunoassays [93].

### *Epitope mapping*

Systematic immunochemical analysis of overlapping, synthetic octapeptides covering the entire coat protein of a potyvirus (JGMV) demonstrated the immunodominance of the surface-exposed N-terminal region of the coat protein (Fig. 8) when intact virus particles are used as immunogens [93]. Antibody responses following two (Scan 1) and five (Scan 2) injections were almost exclusively restricted to the N-terminal 67 amino acid residues with significant responses to the central peptide regions occurring only after prolonged antigen boosting (Scan 3). The immunochemical analysis of the peptides also revealed the location and sequence of the epitopes in the core region of the JGMV coat protein (Scan 4). When overlapping octapeptides equivalent to the six major JGMV core protein epitopes (Scan 4) in seven other potyviruses were synthesized and tested for their ability to bind the cross-reactive JGMV core protein antiserum, their relative contribution to total antibody binding varied between different potyviruses. This is expected as the amino acid sequence in the core protein region of different potyviruses vary (Fig. 1).

The immunochemical analysis of the JGMV octapeptides also revealed the location and sequence of epitopes for two monoclonal antibodies raised against intact particles of JGMV (Fig. 8), one recognizing only JGMV (Mab 1/25) and the other (Mab 1/16) recognizing all 15 potyviruses tested so far (Scans 5, 6). Of great interest was the finding that the epitopes for the virus-specific Mab 1/25 are located in the surface-exposed N-terminal region, and the major region recognized by the cross-reacting Mab 1/16 corresponds to core epitope 1 (Scan 4) recognized by the JGMV core protein antiserum. The latter region has the highest peak of hydrophilicity in the total coat protein and is surface located in native virus particle since it includes the two lysine residues, which mark the junction between the trypsin-susceptible N-terminal region and the trypsin-resistant core (Fig. 1) [93].

### *Outstanding problems in serology*

A major problem with the serology of potyviruses is the presence of unexpected and inconsistent paired serological relationships between viruses which on other grounds can be regarded as distinct potyviruses. Such relationships occur between biologically simlar as well as different potyviruses. There are many such examples in the literature [46, 47, 71, 89, 90, 105]. The serological reactions

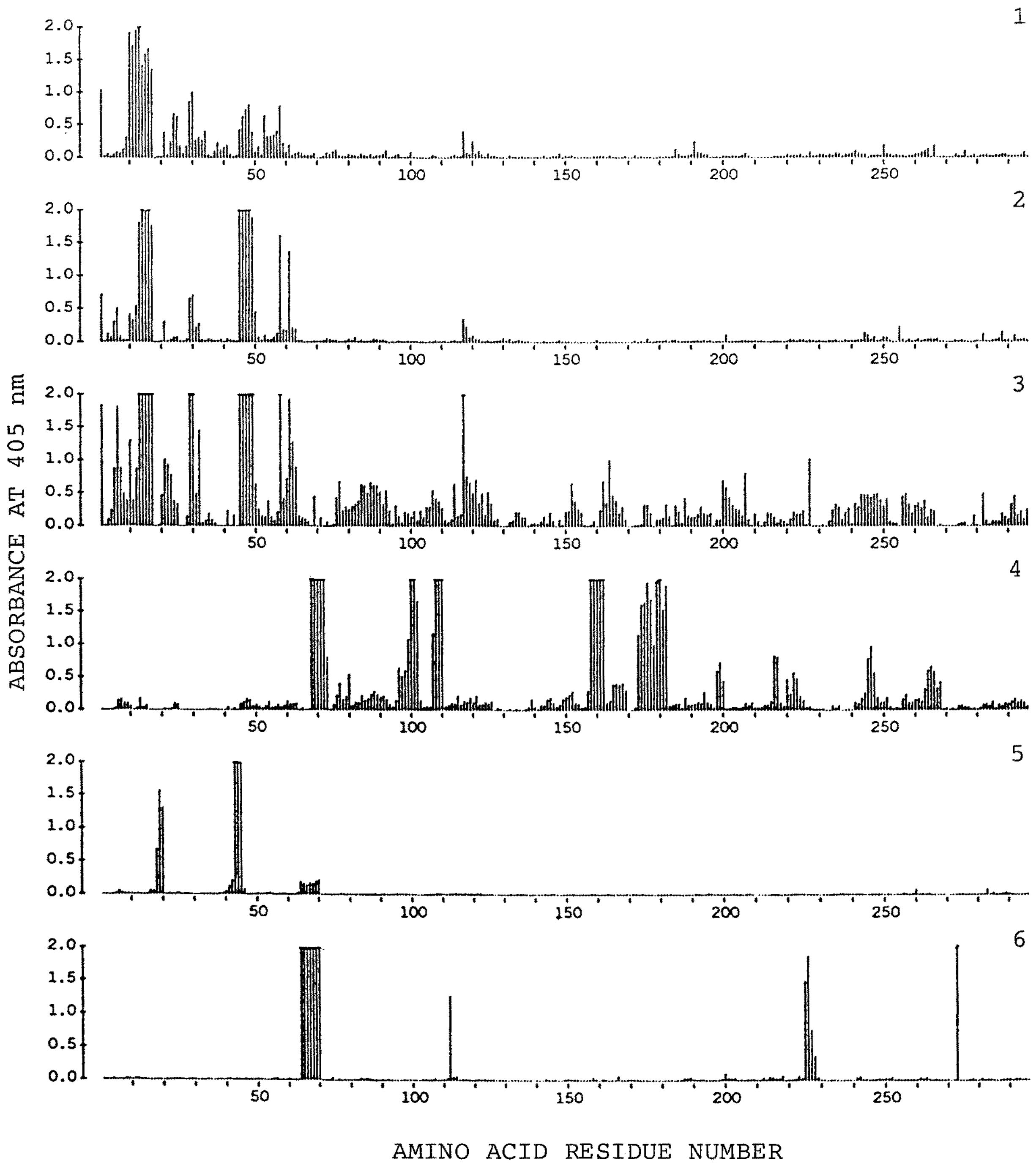


**Fig. 8.** Scans of antibody binding to overlapping, synthetic octapeptides homologous with coat protein sequence of JGMV-JG [85]. The peptides were reacted with polyclonal antibodies to intact JGMV particles from first bleed (**1**), second bleed (**2**), and final bleed (**3**), to denatured JGMV core protein (**4**), and monoclonal antibodies, Mab 1/25 (**5**), and Mab 1/16 (**6**) produced to intact JGMV particles. Adapted from Shukla et al. [93]

obtained with the paired viruses are often similar in strength to the homologous reactions [89, 90].

Investigations of the JGMV and WMV 2 relationship [89] revealed that the epitope for this paired relationship was located in the surface-exposed N-terminal region of the coat protein. Preliminary immunochemical analysis of overlapping, synthetic octapeptides corresponding to the surface-exposed N-terminal regions of JGMV and WMV 2 suggests that the epitope for this paired relationship consists of the first eight amino terminal residues [D. D. Shukla et al., unpubl. results]. Examination of these sequences in the two viruses (Fig. 1) shows that only three of the eight residues (1, 2, and 4) are identical. However, work of Geysen and co-workers [32–34] has shown that only a few amino acid residues in an epitope are key contact residues and are responsible for antibody production and binding; other residues can be substituted without any apparent effect on antibody binding.

The epitopes for other unexpected paired relationships may also reside in this N-terminal region of the coat proteins. In a close analysis of the N-terminal sequences of distinct potyviruses Shukla and Ward [83] observed that limited sequence homology can be found if the N-terminal ends are aligned and major gaps produced. A comparison of the recently determined coat protein sequence of MDMV-B strain of SCMV [J. Jilka and J. M. Clark Jr., pers. comm.] with that of HAT strain of TEV [4] shows that the first eight amino terminal amino acid residues in the two coat proteins are identical, and this region would most probably be responsible for the paired serological relationship between these two viruses [90] as suggested previously [83].

Examination of the known sequences of other potyviruses reveals that they also have common sequences in the N-terminal region as found with the JGMV/WMV 2 and MDMV-B/TEV-HAT pairs. The two strains of BYMV (BYMV-G and BYMV-CS) and BYMV-30 (the latter is not a strain of BYMV on the basis of overall sequence homology) have six of the first eight amino terminal amino acid residues identical [41, 96]. Similarly, Australian strains of BYMV and CYVV were found to have a sequence in the surface exposed N-terminal region where four amino acid residues (residues 6, 7, 8, and 9 in BYMV-G, Fig. 1) were identical in the two viruses [89]. These examples suggest that the problem of paired serological relationships may be more acute with potyviruses infecting legumes than with other potyviruses.

There are more than 10 different potyviruses which cause diseases in leguminous plant species and these viruses appear to form a "continuum" on the basis of their biological and serological properties [7, 10, 16, 58]. It will be interesting to establish whether these viruses possess common sequences in the amino terminal region of their coat proteins which could cause considerable difficulty in serological discrimination. Monoclonal antibodies of defined specificity or polyclonal antibodies to defined synthetic peptides may be required to discriminate these viruses.

A final problem in potyvirus serology is posed by viruses such as PeMV and PVY which are strains of the same virus with very similar coat protein structure yet show only a distant serological relationship [86]. Preliminary results from immunochemical studies of overlapping, synthetic octapeptides corresponding to the N-terminal peptide regions of PVY and PeMV revealed that this lack of serological cross-reactivity is due to a single sequence substitution (in PeMV) at a key contact residue in the major virus-specific N-terminal epitope [R. Lauricella et al., unpubl. results].

## Concluding remarks

The foregoing discussion shows that the classical methods used in the past for the identification and classification of potyviruses do not provide suitable taxonomic criteria which can accurately distinguish distinct viruses and strains. Comparative biological and serological properties reveal a "continuum" implying that the "species" and "strain" concepts cannot be applied to potyviruses [10, 42, 46, 47, 58, 70].

In contrast, the use of coat protein amino acid sequences clearly demonstrated that potyviruses can be divided into species and strains [82]. In addition, the coat protein sequence information in combination with information on the structure of the potyvirus particle [89] can be used to develop simple techniques, such as HPLC peptide profiling [88] and virus-specific antibody probes [90, 92], that are more easily applied than those previously used for potyvirus identification and classification. Immunochemical analyses of native virus particles, trypsin treated virus particles, dissociated core protein [89–92] and overlapping, synthetic octapeptides that account for the entire coat protein [93] have established, for the first time, the molecular basis for potyvirus serology and have explained many of the problems associated with the application of conventional serology to this group of plant viruses.

Coat protein structural information has provided a sound basis for the identification and classification of potyviruses and as a result, the virus/strain status of some potyviruses has been redefined, requiring a change in the potyvirus nomenclature. For example, JGMV has been found to be a distinct potyvirus and not a strain of SCMV [85], the 17 strains of SCMV have been shown to belong to four distinct potyviruses [92], the 13 SMV strains have been reclassified into four distinct potyviruses [82; unpubl. results], and BYMV-CS and BYMV-30 have been shown to be distinct potyviruses [96]. On the other hand, PeMV appears to be a strain of PVY [86] and SMV-N a strain of WMV 2 [31, 109]. These new developments necessitate a re-evaluation of much of the earlier literature on cross-protection and serology. We believe that cross-protection can play a role in the identification and classification of potyviruses provided the reference strain is properly identified and the non-protection results viewed positively.

In spite of these advances, some problems such as the occurrence of unexpected and inconsistent paired serological relationships and single sequence changes in key contact residues in the virus-specific epitopes of some strains (PeMV and PVY) still remain if the identification and classification of potyviruses is based solely on coat protein properties. These problems may be overcome by: the use of monoclonal antibodies to unique epitopes in the N-terminal region of the coat proteins; the use of monoclonal or polyclonal antibodies to defined synthetic peptides; or the use of nucleotide sequence information from other parts of the potyviral genome, including the 3′ untranslated region [31]. While the gene sequence data from the potyviral genome should provide the ultimate criteria for identification and classification of potyviruses, coat protein data is a good index of genetic relatedness, and the coat protein may be a more convenient target for general identification and detection strategies.

Careful thought needs to be given to the new nomenclature required following the re-assignment of viruses and strains based on coat protein/gene sequence relationships. We believe the new name should reflect the new assignment but also permit ready connection to the past literature. For example, SMV-N, now recognized as a strain of WMV 2 [31, 109], should be renamed WMV-SN, i.e., the soybean N strain of WMV 2, with no necessity to repeat the symbols for mosaic or virus. Similarly, WMV 1 should be renamed the WM 1 strain of papaya ringspot virus (PRSV-WM 1) rather than the presently suggested new name PRSV-W [109], and the new nomenclature for MDMV-B and PeMV should be SCMV-MDB and PVY-PeM, respectively.

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