

**Modern Technology Improves Plant Virus Taxonomy
or Melding the Molecular and Classical**

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Identification and differentiation of plant viruses in a manner that allows a useful and logical system of taxonomy has been the desire of plant virologists since the early 1900s. This need was recognized when they realized that two virus isolates causing very similar symptoms on the same plant species can have many other properties that differ.

With the realization that viruses were infectious entities came the discovery of properties that could be used for separation. Some viruses were transmissible by sap inoculation. Some viruses were transmitted by some species of insects (and mites, fungi, or nematodes). Some viruses were pollen or seedborne. Experimental transmission allowed testing of infectibility and type of reactions on a wide range of plant species. Even though these biological properties were more informative than symptoms alone, they still did not provide reliable and accurate differentiation systems for classification. Symptoms formed the basis for several early nomenclatural proposals, but physicochemical properties of viruses led to the understanding that there were indeed different viruses. The first studies were done on the effects of temperature, dilution, and aging on virus infectivity. Such properties of viruses in crude sap were significant in the classification and nomenclature system developed by James Johnson (7).

Direct knowledge of the virus particles gave rise to even more useful schemes of nomenclature and classification, including serological relationships; coat protein size, number, and amino acid composition; genome type, nucleotide composition, size, and segmentation; and particle morphology. Endowing certain properties with more importance than others led to proposals for hierarchical systems of classification (8).

Several groups of viruses were established on the basis of virus properties. Many of these groups have stood the test of time and are as valid today as when they were first proposed. For instance, in 1965, Brandes and Wetter distinguished six groups of elongated virus particles by differences in two characters: particle length and serological relationship (2). The validity of these six groups was confirmed and 10 more groups were formed by a subcommittee of the International Committee on Taxonomy of Viruses (ICTV). These 16 groups were formed by unweighted comparisons of 45 defined virus characters of 99 virus isolates (6).

In spite of the success of this Adansonian approach to virus identification, differentiation, and classification, there were still groups of closely related viruses with characters too ambiguous or difficult to separate, and virologists were hesitant to develop any form of hierarchical system based upon these properties (4). A common statement heard in any discussion about virus taxonomy in the 1970s was that we must wait until virus genome sequences are available.

In 1975 Fiers and associates reported the first complete nucleotide sequence of a virus, the bacteriophage MS2 (3). Currently, there are a number of plant viruses that have been completely sequenced, and many more for which partial sequences are available. Amino acid sequences of virus-coded proteins, either derived from nucleotide sequences or by direct protein sequencing,

are generally preferred for comparisons of virus relationships because they are more stable than nucleotide sequences. Nucleotide sequences of noncoding regions of virus genomes also are important for comparing virus relationships.

Comparisons of virus sequences revealed that, in some virus groups, certain regions of the genome are more variable than other regions. Viruses within a group have a similar gene arrangement and similar replication mechanisms. Different groups of viruses may be related by similar short sequences of specific genes (motifs). Reassortment of genomic components occurs in viruses with multiple partite genomes (more than a single nucleic acid strand composes the genome). True recombination may also occur among RNA genomes of different virus isolates. Sequence information is revealing just how variable viruses really are, as well as exposing affinities among virus groups that had not been previously recognized.

The ICTV used many virus characteristics, both biological and physicochemical, to establish sanctioned plant virus groups. Now, at least one virus in many of these groups has been partially or completely sequenced. The putative polymerase genes of these viruses have been compared, and these comparisons confirm that these virus groups are different, even though there are conserved motifs (5). The ICTV is using information from virus sequences and other characteristics to propose families and genera from the established groups. Again, relationships revealed by amino acid or nucleotide sequences agree with conclusions drawn from the properties of the virus particle and from the biological properties of plant viruses.

While biological and physicochemical properties have been used successfully for grouping plant viruses, interpretation of these properties for distinguishing two closely related viruses within a group may still be difficult. Knowledge of virus sequences is helpful in making separations in these difficult cases. For example, the potyviruses comprise the largest group of plant viruses, with over 150 individual viruses. Members have long, flexuous, rod-shaped particles that are 680–900 nm in length. Cytoplasmic, cylindrical inclusion bodies occur in cells infected by the potyviruses but do not occur in cells infected with viruses belonging to other groups. Although all potyviruses have common properties, other properties of these viruses are dissimilar. For instance, while many potyviruses are transmitted by aphids, some potyviruses are transmitted by fungi, mites, and whiteflies. Also, most potyviruses have monopartite RNA genomes, but a few have bipartite genomes. Comparisons of the coat protein amino acid sequences of these diverse potyviruses revealed that all viruses that produce cytoplasmic cylindrical inclusions are more closely related to each other than to members of other virus groups (9).

Another use of sequence information is verification that several isolates are strains of a single virus. For instance, there are many isolates that resemble potato virus Y (PVY) that cause diseases in many crops. There are other groups of isolates that infect the same crops but cause diverse symptoms. If we know the coat protein or the 3' noncoding nucleotide sequence, we can verify that these isolates are strains of PVY (12) and we can separate isolates of viruses that are very similar to PVY, for instance, pepper mottle virus (13).

Knowledge of virus sequences has provided verification that different viruses are distinct and that isolates of a single virus

are similar. In other words, the species concept has been verified. In one example, Shukla and co-workers made 136 pairwise comparisons among 17 isolates of eight distinct viruses (11). The result was a bimodal distribution curve where isolate comparisons of different viruses had 38–70% homology between sequences, while isolate comparisons of the same virus had 90–99% homology between sequences. This indicates that comparisons of virus sequences can be reliable for establishing relationships among viruses.

Sequence information can be used to separate confusing groups of virus isolates. Some virologists have made one virus group out of a number of sugarcane mosaic virus (SCMV) isolates. Other virologists have persisted in calling some of these isolates maize dwarf mosaic virus (MDMV). Recently, Shukla and co-workers used a number of techniques to separate these isolates into four viruses: johnsongrass mosaic virus, sorghum mosaic virus, MDMV, and SCMV (10). The coat protein sequences of isolates in one virus show close homologies, while isolates of different viruses are considerably less homologous. The segregation of these isolates into the four viruses is consistent with several criteria: 3' noncoding nucleotide sequences, amino terminal serology of the coat protein, coat protein peptide profiles, cytoplasmic inclusion morphology and serology, cell-free translation products, cross-protection, and selected host reactions.

Separation of viruses based on sequence information agrees with a number of other virus properties, even some biological properties. Proper classification of virus isolates is important because diagnostic techniques are no better than the classification system. Correct identification of viruses is important for more reliable breeding for resistance and for selecting proper control measures. Classification can be based upon sequences while routine diagnosis can be accomplished with easier procedures, such as serological assays, selected to agree with sequence separations.

Sequence information also is helping in understanding the relationship between sequences and biological properties; this relationship leads to an understanding of the biological properties that are related to phylogeny. A three-amino acid sequence, ... DAG ..., occurs in aphid-transmitted PVY isolates, but isolates not transmitted by aphids may have a different amino acid sequence, sometimes with a single amino acid substitution in this motif (1). Such a single amino acid change would not represent a series of changes related to its evolution, thus it would not be used in classification.

Most virologists have preferred to avoid taxonomy, considering it an art rather than a science. Now, molecular genetics is helping to explain biological expressions and the relationships of genotype to phenotype. This is leading to a sound taxonomy based on both phenotypic and genotypic characters.

This same phenomena is occurring in many other areas of plant pathology, indeed biology. These are exciting times to be involved in plant pathology, but a thorough understanding of biological

processes is required in order to get the full benefit from molecular genetics. One scientist seldom has the breath of knowledge, understanding, or skills to derive the maximum benefit from both areas. Plant pathologists are uniquely positioned, by training and skills, to be catalysts in bringing the fields of molecular genetics and biology together.

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