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Detection, Identification and Classification of Vegetable Viruses

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Diagnosis is one of the most important procedures in plant pathology. It is essential for the viral diseases, since we have no direct methods to decline the causal viruses. We now have some excellent methods to detect viruses from plant materials. However, it is not easy to identify the causal viruses. We still have difficult problems in plant virus classification.

In this paper I quickly review some methods for detecting or edentifying viruses, those are applicable for vegetable samples. Then, I focus on the problems in plant virus classification. I hopefully show the present status in this field of plant virus research.

Dectection and identification of viruses

Serology is presently a standard technique to detect viruses from diseased plant materials.

Several systems of enzyme-linked immunosorbent assay(ELISA) are very popular. Antigen-antibody reaction in wells of microtiter plates is amplified by the enzyme and density of the solution is measured by a spectrophotometer. Dot-immunobinding assay(DIBA) is a similar system performed on nitrocellurose or mylon membranes. Coloring of the spots is observed without special apparatus. By these methods, plant extract at a concentration of as low as 1ng/ml or 1pg/ml could be detected.

Gel diffusion is another detection method using antisera. It is not practicable to test samples routinely. Nevertheless, minor differences in the antigens can be adequately detected by this method. Sorotypes in one virus species are definithly analyzed by the double diffusion techniaue. Detectativity of this method is lower than those of ELISA or DIBA, but it is improved when Gelrite is used as gelling agent instead of normal agar.

Rapid immunofilter paper assay(RIPA) is a recent technique to detect viruses within a very short time. We developed a new system with colored and noncolored antibody-labelled latex beads which are fixed on glassfiber filter paper strips. Viruses in crude plant sap are detected by dipping the lowed end of the strip just like by way of litmus paper.

We should consider some points when we apply serology to plant virus detection. Positive and negative controls should be set in every serological test. To start serological tests, of course, we need adequate antisera for each method. We can prepare antiserum against purified virus by ourselves or obtain from other researchers or institutions. Antisera may be further purified depend on the techniques.

Some researchers have been trying to use monoclonal antibody(MAb) in practical detection. However, most of the MAbs obtained were strain-specific or isolate-specific. The cocktails, mixture with several MAbs, do not always react with different strains of the same virus. Therefore, polyclonal antibodies prepared in rabbit are preferred in practical diagnosis.

If your institution is equipped with a transmission electron microscope(TEM) and you can it readily, it is very much helpful to plant virus idenification.

Direct negative staining(DN) is an efficient method to observe virus particles such as those of broad bean wilt virus are easily detected in a short time. Certain virus-induced inclusion bodies, pinwheel of potyviruses for example, are also easily detected. Though, loose spherical particles, those of cucumber mosaic virus(CMV) for example, are not detected by the DN.

Immunosorbent electron microscopy(ISEM)is the serology operated TEM support films. It is used to trap increased numbers of virus particles on the grid and also to identify particles by the virus-spcific antibody. Serological direct staining(SDN) is one modification of the ISEM to detect and identify CMV quickly. Intact CMV particles are preserved in the reducing solution and identified by the particle aggregates covered with antibodies.

Nucleic acid hybridization is widely used to identify plant viruses in laboratories. Viral nucleic acids at a very low concentration in plant host can easily be amplified by the polymerase chain reaction(PCR). Though nucleic acid hybridization and PCR procedures are costly, they could be used for limited objectives. Since nucleic acid fragments used as probes or primers are extremely specific, only nuceic acids with matched sequences are detected by these methods.

Diagnosis is the process to identify causal virus for the disease. Therefore, pathogenicity should be confirmed on the inoculated plants. Reproduced sympthoms are reproduces only by the inoculation with multiple viruses.

Present status of plant virus classification

Plant virus clssification is managed through the International Committee on Taxonomy of Viruses(ICTV). The ICTV is a network of virologists under the International Union of Microbiology Societies (IUMS). More than 600 virologists are supporting the activities.

Every researcher could suggest new taxa or any taxonomic alterations for ICTV.
Taxonomic proposals are debated in the Executive Committee(EC). Proposals accepted in the

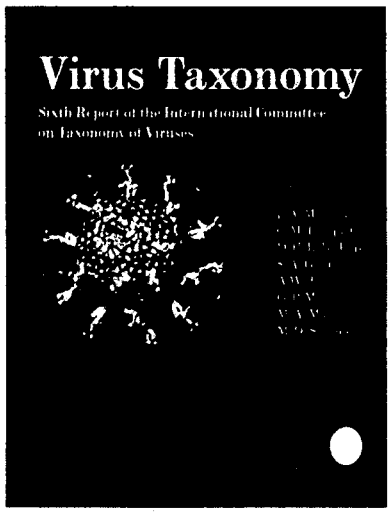
general meeting, which is held every three years, are compiled and published.

Subjects on plant viruses are discusses in the Plant Virus Subcommittee(PVS) which is organized with ten to twenty members from various countries. Consensus is collected through questionnaires from the members and presented to EC by the chair. Present chair of the PVS is M.A. Mayo of U.K. The Japanese PVS member is the only one from Eastern Asia. Various study groups such as the Potyvirus Study Group are placed under ICTV.

In Japan, we have the Committee on Taxonomy of Plant Viruses in the Phytopathological Society of Japam(PSJ), which consists twelve members. The chair of the committee has been occupied by the PVS member. The committee is working for the PVS and also for the subjects in Japan such as determination of the Japanese are listed in "Common Names of Economic Plant Diseases In Japan" serially published by the PSJ.

Classification of plant viruses is also discussed in the International Working Group on Legume Viruses, International Working Group of Vegetable Viruses, the International Working Group of Ornamental Plant Viruses and so on, which are informal networks of researchers. Plant virologists often discuss nomenclature and classification of viruses in meetings for the Food and Agriculture Organization of Unitions (FAO) or other international organizations.

Recent report of the ICTV is the 6th Report of the International Committee on Taxonomy of viruses(1995). From this version of report, the ICTV introduced family-genus system into plant virus classification. Most of the viruses were placed as species status. "Groups" were now changed to genuses. In addition, they did not accept the Latin Binomials for plant viruses. Common names of the plant viruses are still dffective and have been agreed to use as their scientific names.



The 6th ICTV Report contains plant viruses of 429 species in 47 genuses and 10 families. However, 22 genuses are "floating genuses", which have not been classified in families yet. "Archives of Virology" serves as the current official journal for the ICTV.

Another activity operated by the ICTV is constitution of a database of viruses, ICTVdB. All the contents including figures of the 6th ICTV Report are available via Internet at "http://life.anu.edu.au/viruses/ICTVdB/ictvb.html".

The largest database of plant viruses is the Virus Identification Data Exchange(VIDE), which is cooperated by the CAB International. VIDE is accessible at "http://biology.anu.edu.au/research-groups/MES/vide/". VIDE covers more than 900 plant viruses. Printed version of VIDE

was published by CAB International in 1996.

Problems and prospects

One of the problems in plant virus classification at present is uneven minuteness among various taxa. Small differences resulted in many different species in *Potyvirus*, whereas considerable diversity is included in one species in *Tobamovirus*. Many genuses are still"floating" and their status has not been fixed. We have to prepare families or orders to complete the system. Nucleic acid research will soon provide certain information for it.

The 6th ICTV Report suggested a profound definition for a virus species as follows; "A virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." This definition is rather difficult, however, it is desirable to express a virus as a fuzzy set.

Since a virus species is understood by this definition, we should consider a virus as a population including minor variations. Therefore, viruses should not be identified by a single characteristic such as MAb or infectivity to one host. It is especially important to identify viruses by nucleic sequences. PCR research must be conducted for at least a number of clones.

I have to point out the significance of bioassay once more. Viruses in plant pathology are important since they are the causal agents for diseases. We should not skip bioassay, although it is not easy work.

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