

DIAGNOSING PLANT VIRUS DISEASES: Approaches suited to available technology



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What are Viruses?

- ❑ Sub-microscopic infectious particles (virions) composed of a protein coat and a nucleic acid core.
- ❑ Carry genetic information encoded in their nucleic acid, which codes for two or more proteins
- ❑ Replication takes place within the host plant cell, but some may multiply in vectors e.g aphids and nematodes i.e are not functionally active outside their hosts.
- ❑ Host specificity; most viruses are restricted to a particular type of host. A few have very wide host ranges.

Why are viruses important?

- Viruses cause many diseases of National, regional and international importance.
- Viruses are responsible for huge losses in production and quality
- Infected plants may show a range of symptoms depending on the disease such as;
 - leaf yellowing either of the whole leaf or in a pattern of stripes or blotches
 - leaf distortion e.g. curling
 - growth distortions e.g. stunting of the whole plant
 - abnormalities in flower or fruit formation.

Classification of viruses

Many different characteristics are used to classify viruses into families, genera and species. Typically, a combination of characters are used and some of the most important are:

1. **Particle morphology**: the shape and size of particles as seen under the electron microscope.
2. **Genome properties**: this includes the number of genome components and the translation strategy. Where genome sequences have been determined, the relatedness of different sequences is often an important factor in discriminating between species.
3. **Biological properties**: this may include the type of host, host range and also the mode of transmission.
4. **Serological properties**: the relatedness (or otherwise) of the virion protein(s).

Why virus diagnosis/identification?

Accurate diagnosis combined with early detection of plant viruses is critical for effective management of diseases.

- For plant quarantine, it is necessary to ensure that plant material is free of quarantine viruses before release to industry or breeding programs.
- In nuclear stock production programs, virus detection ensures freedom from viruses.
- Epidemiological studies require sensitive tests in which a large number of samples can be tested quickly
- Surveillance and monitoring

Attributes of a good diagnostic technique

- ❖ Accurate
- ❖ High specificity
- ❖ High sensitivity
- ❖ High speed

Diagnostic Techniques

- Symptomatology
 - indicator plants
 - host range
- Serology
- Electron microscopy
- Nucleic acid-based methods
 - PCR
 - Nucleic acid hybridization methods

Symptomatology and Host range

Symptoms are the initial step in disease diagnosis. On their own however, are insufficient because;

- symptoms may result from infection of more than one virus.
 - Different viruses may individually cause the same symptoms in the same host
 - Different strains of the same virus may individually induce different symptoms on the same host.
 - Symptoms may vary with the cultivar of the host
 - Symptoms may be influenced by the environmental conditions
 - Many plants are carriers of plant viruses but show no disease
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- Some symptoms indicate that the infecting virus belongs to a particular group of viruses.
 - Some viruses have a specific host range which may greatly assist in their identification.

Symptoms



Yellow mosaic symptoms on lettuce
Caused by lettuce mosaic virus



SPVD



Yellow vein-banding -caused by
Grapevine fanleaf virus

Typically, virus infections of plants might result in effects such as growth retardation, distortion, mosaic patterns on the leaves, yellowing, wilting **etc**

Use of herbaceous indicator plants in virus detection



Most SP cultivars show no symptoms
Detection in sweet potato is not sensitive in ELISA/PCR
Viruses are readily detected in ELISA following graft inoculation;
symptoms are also conspicuous in indicator plants.

Experimental host ranges of SPFMV strains

Test plant	KY-46b	Zambia	Rak6e	115/1S	25/4A	MD1/1
<i>N. benth.</i>	+	+	-	-	-	-
<i>N. hesperis</i>	+	+	-	-	-	-
<i>N. occid.</i>	+	+	-	-	-	-
<i>N. clevel.</i>	+	+	-	-	-	-
<i>C. quinoa</i>	+	+	+	+	-	-
<i>C. amarant.</i>	+	+	+	+	-	-
<i>C. murale</i>	+	+	+	+	-	-
<i>I. setosa</i>	+	+	+	+	+	+

Transmission of Plant Viruses

Plant viruses vary in their mode of transmission.

A few viruses are transmitted by more than one mode;

- **Arthropods: Insects - aphids, leafhoppers, planthoppers, beetles, thrips, etc.**
 - Non-persistent transmission
 - Semi-persistent transmission
 - Persistent transmission
- **Fungi**
- **Nematodes**
- **Mites**



- **Sap transmission is important for only a few viruses and occurs on implements, hands of workers, and clothing.**
- **Pollen transmission from male flower to female occurs for a few viruses. Such viruses are seed-borne**
- **Graft transmission/transmission by dodder – limited to experimentation**

Mode of Transmission

- Sap transmission and the alternative modes of transmission may help in diagnosis
- Transmission of a virus by a specific vector e.g nematode transmitted nepovirus immediately indicates the virus under investigation.

Serology

Serology may be done to detect viruses on

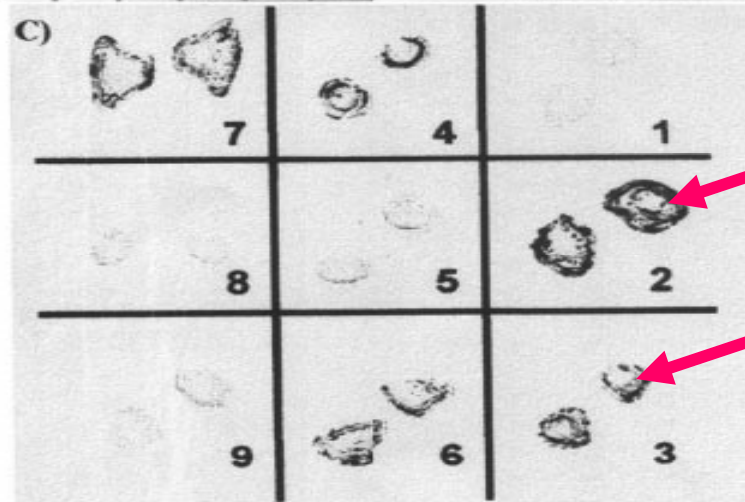
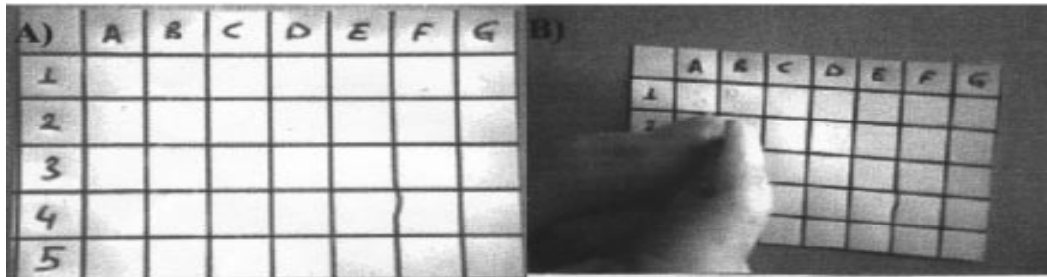
- Plant sap
 - In virus vectors
 - Natural reservoirs e.g weeds
 - Purified virus preparations
- ELISA* represents a very sensitive serological method to detect viruses in extracts from infected plants.

* Enzyme-linked immunosorbent assay

Some ELISA formats

- Double antibody sandwich (DAS-ELISA)
- Triple antibody sandwich (TAS-ELISA)
- Nitrocellulose membrane (NCM-ELISA)
- Plate trapped antibody (PTA ELISA)
- Tissue Blot immuno Assay (TBIA)
- On-site testing kits: usually simple commercial kits for rapid large numbers detection of relatively large numbers of samples; can be done by relatively by non-experienced personnel
e.g immunostrip assays

Tissue Blot immuno Assay



Electron Microscopy

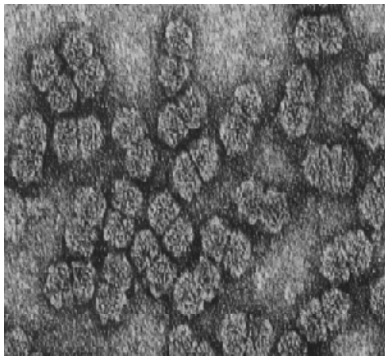
An electron Microscope uses a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield the following information:

1. Topography
 - The surface features of an object "how it looks",
2. Morphology
 - The **shape and size** of the particles making up the object
3. Composition
 - The elements and compounds that the object is composed of and the relative amounts of them;
4. Crystallographic Information
 - How the atoms are arranged in the object; direct relation between these arrangements and materials properties

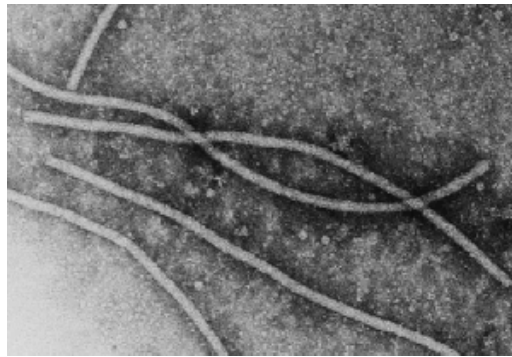
EM: Historical Background

- ⇒ Originally, techniques were readily available for purified virus preparations only.
- ⇒ The use EM for detecting viruses in crude sap was reported in the 1950s
- ⇒ Use of the EM for the detection of plant viruses that occur in relatively high concentration and have elongated or rod-shaped particles is a quick, easy, reliable procedure through the 'leaf dip' technique.

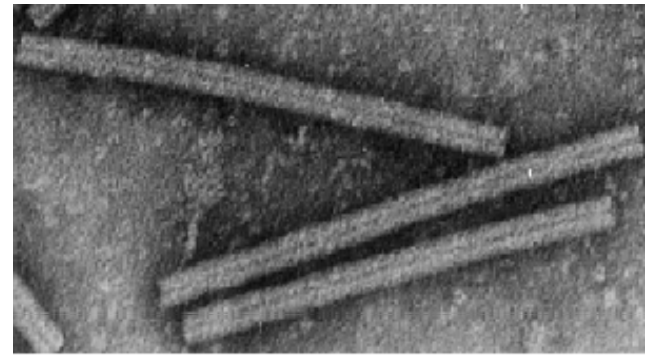
Some Shapes and sizes of viruses may be diagnostic



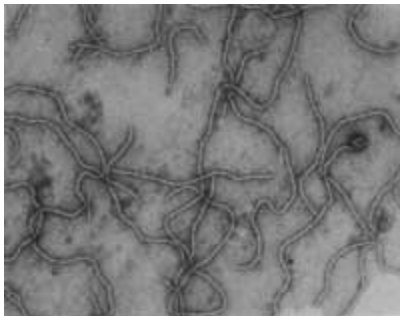
Geminate



Filamentous



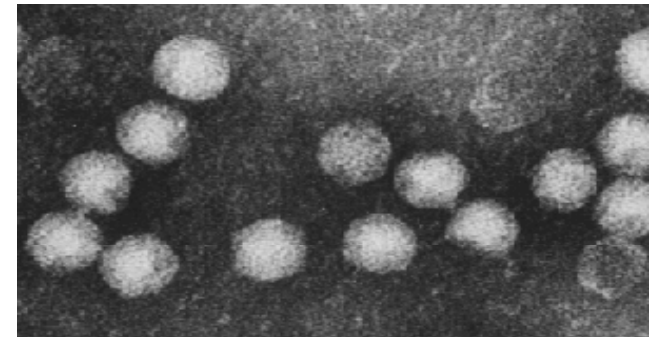
Rod-shaped



Highly flexuous



Bullet-shaped



Spherical

Direct EM

- Can be a quick and sensitive approach to diagnosis of virus diseases
- Allows for recognition of particle size & morphology.
- Grids are floated on crude extracts for 5 min, washed in several drops of distilled water and negative stained with 5 drops of 1% Uracyl acetate.
- The particle morphology and fine structure of a virus can be recognized (at X 40,000)

Immunosorbent EM (ISEM)

Combines serology and electron microscopy

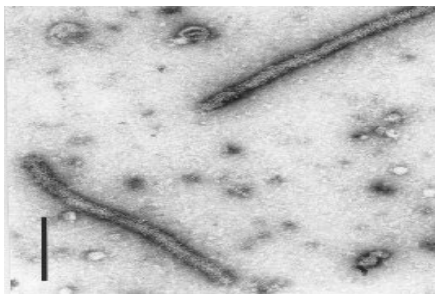
- Grids are floated on 1:1000 diluted antiserum for 5 min
- Grids are washed with 20 drops buffer
- Grids are then incubated on a drop of plant extract for 15 min to several hrs
- Grids are washed with several drops of dH₂O
- Grid stained with 3 drops 1% Uranyl acetate.

Applicable where virus particle concentration is low; the antiserum therefore traps the particles.

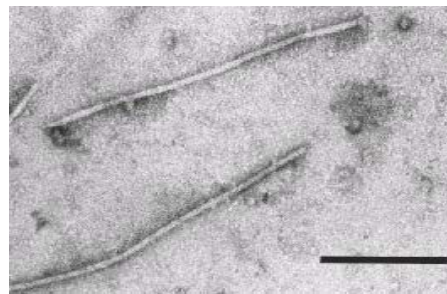
ISEM and Decoration

- After incubation of virus samples for trapping, a decoration step can be done using crude sera.
- The step is important for the evaluation of the decoration of low concentrated viruses with homologous or heterologous antisera

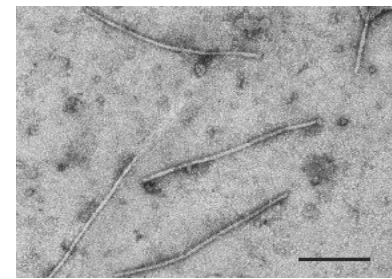
E.M and serological differentiation between SPVY and SPFMV



**SPVY particles
with homologous
antiserum**



**SPVY particles
incubated with
SPFMV antiserum**



**SPFMV particles
incubated with SPVY
antiserum**

PCR

- Nucleic acid based detection systems were greatly improved following the development of the polymerase chain reaction (PCR).
- This assay is relatively easy to perform but it is still not amenable to handling large numbers of samples
- Synthesis of group-, virus-, or strain-specific primers requires nucleotide sequence information for at least several members of a virus group
- PCR is able to detect pathogens by targeting their genetic material (RNA/DNA)

PCR Formats

- PCR
- RT-PCR -For RNA viruses
- IC-PCR -For DNA & RNA viruses
- IC-RT-PCR -For RNA viruses

Immunocapture PCR combines the advantages of serology and PCR into a very sensitive method of detection

Multiplex PCR allows for simultaneous and sensitive detection of different DNA/RNA in a single reaction

Real time PCR combines amplification, detection and quantitation or quantification in a single step.

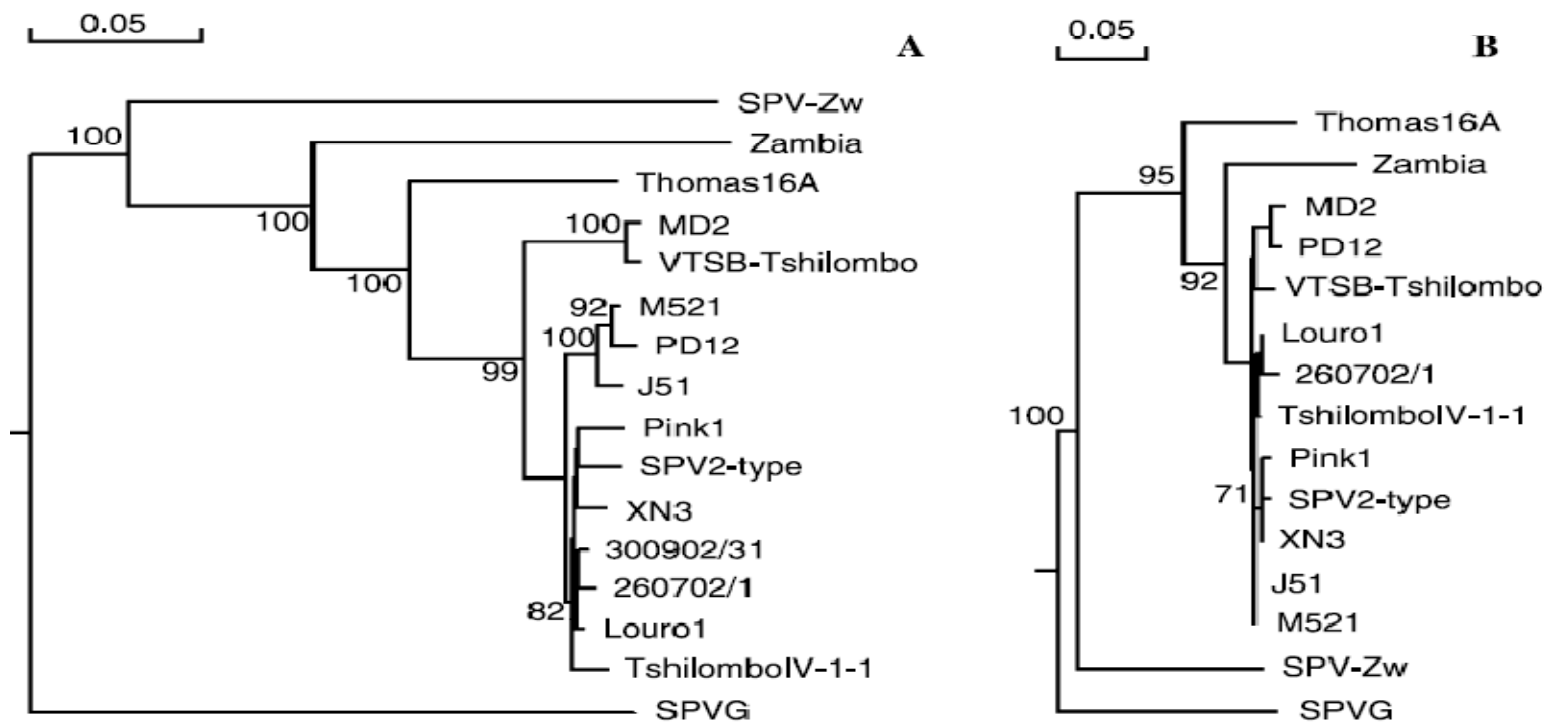
IC-PCR

- Immunocapture employs serology to selectively trap virus particles to the tube wall which has been pre-coated with virus-specific antibodies. Other substances such as phenols that inhibit PCR are washed away.
- This way, a suitable template for RT and/or PCR are obtained
- IC is similar to the coating step in DAS ELISA.
- After incubation, the tube is washed and subjected to RT/PCR step.

Sequences and species/strain delineations

- Genome organization
- Sequence similarity and species and strain demarcation

Species/strain resolution: Phylogenetic analyses



Microarrays

- Microarrays are one of the new emerging methods in plant virology
- DNA microarrays or biochips are made of a surface on which multiple capture of DNA sequence of the targets is possible.
- The purpose is to detect numerous sequences in a single assay
- Used at research level

Choice of detection technology

The choice of a virus detection technique depends on several factors;

1. The reason for which the test is required,
2. The availability of high quality antisera, cDNA probes or oligonucleotides to provide the desired specificity,
3. Available knowledge of the virus to be detected and number of samples to be tested.
4. The available expertise is also an important consideration.
For someone very familiar with the use of cDNA technologies but with little or no experience with serology, the method of choice will likely be a cDNA-based technology.

Nucleic acid based methods or Serology?

- Each technology offers advantages over other methods of detection but at the same time each has its limitations.
- Depending on the requirements of the assay, either ELISA or a nucleic acid-based technology may be the method of choice.
- In some instances, where confirmation is essential, a combination of two methods may be required.
- In other cases neither of these technologies will be satisfactory and it may be necessary to use grafting or mechanical transmissions to herbaceous indicator hosts.
- Monoclonal antibodies, nucleic acid hybridization and PCR have resulted in the development of diagnostic reagents with high specificities.

Challenges/considerations

- Lack of sensitivity is a problem in recently infected plants no matter how good the test
- Vectors that have acquired virus several weeks before testing, or have only fed on an infected source plant for a short time may contain a virus concentration that is below detectable levels.
- Unavailability of the necessary equipment to perform the various detection assays.
- Need to develop diagnostic reagents for many plant viruses (antisera)
- Application of these technologies to recalcitrant viruses.

Food for thought!

An important consideration when using diagnostic tests that are not based on biological activity is the significance of the result.

For example, in PCR a positive "test" for a pathogen DNA does not show that the pathogen is living. It only shows that the pathogen DNA was present in a sample.

The legalities of a decision to turn back a shipment of plant product based on a positive laboratory test result not based on biological activity is an issue that is debatable.

What should a decision be based on?

Summary

- There are a variety of laboratory assays for virus detection available
- These assays differ in sensitivity, specificity and appropriateness of use
- Many new tools and technologies are available. Cost and labor needs are significant factors in selection.
- All techniques and assays may need validation before use in routine testing.

Thank you for listening

