Plant virus Transmission

Pl. Path. 502  (Cr. Hrs. 2+1)

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Transmission is fundamental property of viruses

Plant viruses being obligate parasites must be spread from one susceptible host to another and need to be introduced in living cell for their survival and continuity.
The knowledge of virus transmission is important to:

- Recognize a virus as cause of the disease if transmitted from infected to healthy plant
- How virus spread in field – help in its control
- Establish biological relationship of interaction between virus and its vector
- Mechanical transmission is very important for lab. Study of viruses.
There are two types of plant virus transmission:

- **Horizontal transmission**
  - Horizontal transmission is by vectors, human pruning shears and tools, and other direct, external contamination.

- **Vertical transmission**
  - Vertical transmission occurs when a plant gets it from its parent plant. Either through asexual propagation (cuttings) or in sexual reproduction via infected seeds.
Methods of transmission

- Non-Insect transmission:
  - Sap inoculation/ Mechanical: TMV, PVY
  - Seed: BCMV,
  - Fungi: Olpidium brassicae- TNV
  - Vegetative & graft transmission: PVY, PLRV, Fruit viruses
  - Nematodes: Xiphinema index: Grapevine fan leaf virus
  - Dodder: CMV, TRV

- Insect Transmission: PVY, CMV, BGMV
Mechanical Transmission

- Occurs when plant come in contact with other plant and leaves rub together
- By the action of humans
- Mechanical transmission involves the introduction of infective virus or biologically active virus into a suitable site in the living cells through wounds or abrasions in the plant surface
  - *This method is generally used for experimental purposes under laboratory conditions* - also known as **Sap inoculation**
Methods

- Leaf rub
- Cotton swab
- Pinprick
- Microinjection

Steps:
- Sap extraction
- Extraction medium
- Use of additives
- Choice of suitable host: most common are *Nicotiana* spp., *Chenopodium* spp., *Cucumis sativus*, *Gomphrena*, *Datura* spp, *Phaseolus vulgaris*
Factors affecting mechanical transmission

- Source & preparation of Inoculum
  - Leaf - most common source
    - Have high conc of virus
  - Roots: TNV
  - Fruits, flower & pollens
- Symptoms expression
- Virus concentration
Factors affecting mechanical transmission

- Extraction medium (Water & Buffer)
- Buffers (Po$_4$, borate, citrate, Tris-HCL)
  - Retain infectivity
  - Stability
  - Intact virus
  - Avoid aggregation
- Buffer pH: 7-8 pH
Metal ions and ionic strength
- Some viruses require divalent metal ions (Ca\(^{2+}\) or Mg\(^{2+}\)) for retention of infectivity and structural integrity
- pH, chelating agents and ionic strength help in virus stabilization in extracted sap.

Chelating agents eg. EDTA
- Help in removal of host ribosomes; avoid virus aggregation; prevent oxidantion of polyphenols

Reducing agents: (Thioglycolic acid, Ascorbic acid, cysteine hydrichloride sodium sulphite, 2-mercaptaethanol)
- These prevent oxidation of plant extract and preserve infectivity of the virus
Factors affecting mechanical transmission

- **Substances protecting against phenolics:**
  - cysteine hydrichloride, sodium sulphite: prevent action of phenol oxidases.
  - PVP-polyvinyl pyrrolidine, PEG: reduces binding of virus with phenols

- **Additives that removes plant protein and ribosomes**
  - Mg bentonite- reduces contamination of virus extract with nucleases and ribosomes (mainly 19s protein)
  - Charcoal: adsorb host pigments
  - Na EDTA @ 0.01M ph 7.4

- **Enzymes:**
  - eg. Pectinase is used to degrade mucilage in sap of cocoa leaves prior to precipitation of CSSC, Trypsin -TuMv
Factors affecting mechanical transmission

- **Detergents & other additives**
  - Non-toxic detergents like Triton X-100 or Tween 80 – used in extraction medium help in release of virus particles from cell components

- **Application of inoculum**
  - Use of abrasives
  - Cotton swabs, leaf rub
  - Pressure
  - Washing of inoculate leaves
Factors affecting mechanical transmission

- **Host plant**
  - Stage
  - Choice of host
  - Susceptibility
  - Nutrition

- **Environment**
  - Light
  - Temperature
  - Time of day
  - Time of year - depends on virus-host
Many (if not most) plant viruses have evolved specialized movement proteins which modify the plasmodesmata.

One of the best known examples of this is the 30k protein of tobacco mosaic virus (TMV). This protein is expressed from a sub-genomic mRNA and its function is to modify plasmodesmata causing genomic RNA coated with 30k protein to be transported from the infected cell to neighbouring cells.

Other viruses, such as cowpea mosaic virus (CPMV - Comovirus family) have a similar strategy but employ a different molecular mechanism. In CPMV, the 58/48k proteins form tubular structures allowing the passage of intact virus particles to pass from one cell to another.
Vegetative & Graft Transmission

- **Aim**: is to establish organic union between the cut surfaces of tissues of two different hosts
  - Shoot-Scion
  - Root bearing portion - stock
- **If either stock or scion is infected the virus usually moves to the healthy portion and express symptoms**
- **A pre-requisite for successful graft transmission is the perfect union of the cambium layers of the stock and the scion.**
- **Eg. CTV, Apple mosaic virus etc.**
Method of grafting

- T-budding
- Cleft grafting
- APPROACH GRAFT
Dodder transmission

- *Cuscuta* spp. (Benett (1940))
  - About 20 spp.
  - *C. campestris* & *C. subinclusa* are common
  - E.g. Phytoplasma

- Vegetative propagations
  - Cuttings
  - Tubers
  - Corms etc.
  - Bulbs
Cuscuta
Insect transmission

- **Vector:** various biological agents which introduce the virus into plant tissue are called vectors.
- **Insects** – most important group
  - > 400 spp. have been reported to transmit plant viruses.
  - 94% of these belong to Phylum Arthropod in which 90% are insects.
  - 70% of insects belong to Homoptera in which Aphididae are the most important group.
  - Others: leafoppers, plant hoppers, whiteflies, beetles, mealy bugs, thrips, mites
- **Others:** fungi, nematodes
- **In general,** plant viruses transmitted by one group of vectors are not transmitted by other group except: TRSV which can be transmitted by nematodes and by thrips & spider mites.
Terminology use in virus transmission

- **Acquisition access period**: time for which a initially virus free vector is allowed to access a virus source and could if it desire feed on that source.

- **Acquisition feeding period**: time period necessary for successful acquisition of the virus by its vector which then become viruliferous.

- **Inoculation access period**: time for which a virus carrying vector is allowed to access a virus free plant and could feed on it.

- **Inoculation feeding period**: time period for which a virus carrying vector appears to be feeding on a virus free plant to transmit it.
- **Transmission threshold or inoculation threshold or Inoculation access threshold:**
  the minimum initial time period that a vector need to acquire a virus and inoculate it to the virus free plant

- **Infective capacity or retention period of vector:**
  time period for which a vector carries/ retain/ transmit the virus to host plant and remain viruliferous.

- **Incubation period or latent period:**
  The time period from the start of acquisition feeding period until the vector can infect the healthy plant with the virus.
Virus vector relationships

- Watson & Roberts (1939) gave the basic concept
  - Based on virus retention time by the vector
  - Non-persistent
  - Persistent
  - Sylvester (1958) introduced term Semi-persistent

- Virus vector relationship is also based on
  - Site of retention of the virus in vector
    - Stylet borne
    - Circulative
    - Propagative
    - Transovarial transmission
Non-persistent Viruses

- Such viruses are acquired by the vector during probing and feeding on host parenchyma including epidermal cells.
- Probing takes as little as 5 seconds.
- Vector becomes infective immediately after feeding.
- Virus lost by the vector during moulting.
- No latent period.
- Such viruses are mechanically transmissible.
- Acquisition fasting increases acquisition of virus and transmission.
- E.g. CMV, BCMV, PVY, PSBMV, PRSV, PMV.
Non-persistent Viruses
Semi-persistant viruses

- Virus persist in its vector for 10-100 hrs.
- Acquired from phloem region with long feeding
- No latent period
- Do not circulate and multiply in its vector
- Infectivity lost in moulting
- Particles accumulate at special sites
- High vector specificity
- E.g. CTV, CaMV, BYV
Persistent viruses

- Virus persist in their vector for >100 hrs and in some cases for whole life of vector
- Virus multiply and circulate in vector body
- Latent period is present
- Moulting has no effect of virus

After virus uptake → alimentary canal → gut wall circulate In the body fluid (Haemolymph) → salivary glands causing contamination of saliva → transmission

Also called as
- Circulative
- Circulative propagative
- Trans-ovarial transmission

E.g. PLRV, RDV, PYDV, BYDV
persistent transmission: (syn. circulative transmission) a type of virus transmission in which the virus is acquired and transmitted by the vector after relatively long feeding times and remains transmissible for a prolonged period while in association with its vector aphid vector feeding on a plant host showing the internal route of the viruses that cause barley yellow dwarf)
Circulative transmission: (syn. persistent transmission) virus transmission characterized by a long period of acquisition of the virus by a vector (typically an insect), a latent period of several hours before the vector is able to transmit the virus, and retention of the virus by the vector for a long period, usually several days; the virus circulates in the body of the vector (aphid vector feeding on a plant host showing the internal route of the viruses that cause barley yellow dwarf)
Aphid as virus vector

- Most important group of vectors; >370 spp. Transmit > 300 viruses

*Myzus persicae*: most efficient among all; transmit >100 viruses

Apterous and winged forms
### Features of aphid transmitted viruses

<table>
<thead>
<tr>
<th>Feature</th>
<th>Type of persistence</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Non-persistent</td>
</tr>
<tr>
<td>Mechanical</td>
<td>+</td>
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<tr>
<td>Common symptoms</td>
<td>mosaic</td>
</tr>
<tr>
<td>Tissues of virus acquisition</td>
<td>epidermis</td>
</tr>
<tr>
<td>Fasting effect</td>
<td>=</td>
</tr>
<tr>
<td>Acq. time</td>
<td>Seconds to min.</td>
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<tr>
<td>Latent period</td>
<td>-</td>
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<tr>
<td>Retention of virus through moultng</td>
<td>-</td>
</tr>
<tr>
<td>Vector specificity</td>
<td>low</td>
</tr>
<tr>
<td>examples</td>
<td>CMV, PVY</td>
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</tbody>
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Helper virus:

- These are the viruses transmitted by aphid vectors under certain conditions.
- A aphid transmit can transmit the virus only if the source plant is infected by second virus.
- So it is a **dependent virus** and second virus is referred as the **helper virus**

<table>
<thead>
<tr>
<th>virus</th>
<th>Helper virus</th>
<th>vector</th>
<th>Type of transmission</th>
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</thead>
<tbody>
<tr>
<td>Potato aucuba mosaic virus</td>
<td>PVA or Y</td>
<td>M. persicae</td>
<td>Non persistent</td>
</tr>
<tr>
<td>PVC</td>
<td>PVY</td>
<td>M. persicae</td>
<td>Non persistent</td>
</tr>
<tr>
<td>Carrot mottle</td>
<td>Carrot red leaf</td>
<td>Cavariella aegopodii</td>
<td>Semi persistent</td>
</tr>
<tr>
<td>Tobacco mottle</td>
<td>Tobacco vein distortion</td>
<td>M. persicae</td>
<td>Persistent</td>
</tr>
</tbody>
</table>
White fly:

- Transmit rugose diseases causing mosaic to leaf distortions
- *Bemisia tabaci*
  - Virus vector relationship mostly circulative; semi persistent to persistent
  - Females efficient in transmission
  - LP-few hrs.
- *Phloem*
  - Virus not transmitted by sap
  - TLCV, MYMV, CICV, BYVMV, BGMV, SYMV
Bemisia tabaci

Nymphs

Adults
Leaf hppoers/ plant hoppers

- > 30 viruses
- Semi & persistent transmission
- Propagative viruses
- Causes mainly yellows, leaf rolling
- Phloem cells
- Leafhopper:
  - RDV: *Nephotetix cincticeps*
  - Rice tungro: *N. impicticeps*
  - Transovarial transmission: RDV
- Plant hopper: maize mosaic: *Pergrinus maidis*
- Tree hopper:
  - Tomato pseudo curly top: *Micrutalis malleifera*
Hoppers: *Micrulalis malleifera*, the treehopper vector of *Tomato pseudo-curly top virus*
Thrips: transmit viruses in the genus *Tospovirus.*
Mites (*Aceria* spp.): Transmit viruses in the genera *Rymovirus* and *Tritimovirus*. *Aceria tosichella*, the vector of *Wheat streak mosaic virus* (*WSMV*). Pigeon pea sterility mosaic: *Aceria cajani*
Beetle transmission

- Acq. Feeding: upto 24 hrs
- Persistent transmission
- Cowpea mosaic virus: *Ceratoma trifurcata*
- Turnip yellow mosaic: *Phyllotreta sp.*
Nematode transmission

- Nematodes as vectors of plant viruses initiated research in Nematology and Virology Hewitt et al. (1958)
- Helped in understanding of the transmission and etiology of an important group of soil-borne plant virus diseases.
Nematode transmission

- Two single-stranded RNA virus genera, Nepovirus (NEPO) and Tobravirus (TOBRA), have nematode vectors
  - Nepoviruses: Comoviridae family
  - Tobraviruses: family not yet assigned
Tobraviruses

- Tobraviruses (Tobacco Rattle viruses) have nematode vectors (Lamberti and Roca, 1987).
- Tobraviruses are straight tubular particles with two size ranges, 180-210 nm and 45-115 nm. *Trichodorus* and *Paratrichodorus* are vectors.
Nepoviruses (Nematode-transmitted Polyhedral viruses)

Nepoviruses are isometric (polyhedral) particles around 30 nm in diameter (1 nm=10^-9 m, 1 µm=10^-6 m). The only known nematode vectors are in the genera *Xiphinema* and *Longidorus*. 
Vector Specificity

- 11 spp of *Xiphinema* transmit 13 NEPO viruses.
- 11 spp of *Longidorus* transmit 10 NEPO viruses. 14 spp of *Trichodorus* transmit various strains of two TOBRA viruses: tobacco rattle and pea early browning.
- Lamberti (1987) suggests that since several Trichodorid spp. transmit the same virus, and that both viruses are transmitted by the same nematode, vector specificity is less developed in *Trichodorids* than in *Longidorids*.
• Transmission Characteristics

Trichodorids may retain the virus for up to a year. Acquisition time may be less than an hour to several days, depending on the feeding characteristics of the nematode.

Retention sites:

*Longidorus* - *odontostyle* area

*Xiphinema* - *odontophore* and esophagus region

*Trichodorus* - onchiostyle and esophagus.
Odontostyle

- **odontostyle** is the protrusible hollow or grooved spear or tooth. It appears to form in a cell that is esophageal in position, but that cell is one that has probably migrated from the stoma region.

- **odontophore** is a cuticular extension of the spear that is esophageal in origin - from the esophastome.

- **odontostyle** often distinguished by referring to it as the spear.
Transmission by fungi:

- **Teakle 1960**: TNV transmitted by fungus *Olpidium brassicae*

- **Barley yellow mosaic virus**: Transmitted by the plasmodiophorid fungus *Polymyxa graminis* *(Kusaba et al., 1971; Adams, 1990a)*, which is an obligate root parasite.
Polymyxa graminis: the vector of several cereal viruses including e.g. *Bymovirus* *Benyvirus*, *Furovirus*, *Pecluvirus* and *Pomovirus*

The virus is acquired when the plasmodia of the fungus are growing inside the barley root cells and it is transmitted within the zoosporangia or resting spores that it produces.

*Transmission by fungi:*

Light micrograph of barley root stained with methylene blue, showing plasmodium of *Polymyxa graminis.*
Zoospore suspension of *Polymyxa graminis*.
Polymyxa graminis: Resting spore clusters in infected barley root single cluster.

- The virus survives within the resting spores and infectivity has been retained in air-dried soil for 5-10 years.
Specificity of transmission by vectors

Why the vector of most viruses are confined to one taxonomic groups?

Why TMV is not transmitted by homoptera and why different strains of a virus may differ in vector transmissibility?

No definite answers are there but indicate some factors involved with this phenomenon.

Transmission is the outcome of sequences of processes
  - Acquisition of virus by vector, survival of infectivity during association of virus and vector,
  - Inoculation of virus particle to plants followed by the initiation of infection

Failure of any one of these processes will result in no transmission
Various possibilities are

- Non-vector may fail to acquire virus because it does not feed on the appropriate tissue
- Plant spp. That are host of the virus may not be host of the non-vector
- Virus particles may be taken up by non-vector but not transmitted
- In case of fungi and nematodes vectors there may be specificity for the attachment of the virus which may depend on some property of the protein coat of the virus particle.
- There may be a transmission factor which may be absent in non-vector
- Virus coat protein seems to play a critical role in vector specificity especially with viruses that do not multiply in their vectors.
Aphid saliva may also influence the transmission efficiency and specificity

- During probe, the PPO activity of the sheath saliva may affect the virus
- And at the same time physiology of the host cell may be affected by the salivary phenols and phenolases and CHO hydrolyzing enzymes.

A comprehensive understanding of biology of vector transmission processes may reveal the elements in the transmission sequence which determine vector specificity and efficiency.
Bimodal transmission

- While aphid transmit non-circulative viruses either in non-persistent or semi-persistent manner
- Few viruses have been known to be transmitted in both the manners
- This typical mode of transmission was first referred as bimodal transmission by Chalfant and Chapman (1962) in case of CaMV by *M. persicae* and *B. brassicae*. 
Seed Transmission:

- About $1/7^{th}$ of the viruses transmitted through seed
- Effective mean of introducing virus into crop at early stages with number of infection loci.
- Help in dispersal across area
- Economically important
- Help in virus survival
Seed Transmission:

- **Seed transmission occurs in two ways**
  - Externally seed borne
    - due to external contamination of the seed with virus particles (TMV, PVX)
  - Internally seed borne (BCMV, CMV, BYMV, ULCV)
    - due to infection of the living tissues of the embryo.
    - Virus may be found in different parts of the seed but generally in embryonic tissues

- The embryo become infected by two routes
  - Directly from mother plant
  - By pollens
    - Developing embryo can be infected before fertilization by the infection of the gametes or by direct invasion of the embryo after fertilization

- Virus moves through the testa of immature seed after fertilization and reach micropylar region for embryo infection to occur. Micropyle is in close contact with the base of embryonic suspensor that help in nutrient flow to embryo.
Transmission due to surface contamination of seed or viruses in the seed coat is rare and found in viruses like TMV.

Most of transmission occurs when the embryo becomes infected.

The virus is distributed at random and can be found in different parts of seed:
- Embryo: BCMV, CpMv, TRSV
- Endosperm: BSMV
- Seed coat: BCMV, TMV, TSWV
Dicot seed

Seed and germination

Embryonic:
Root
Shoot
Leaves

Cotyledon

Bean Cut in Half
Monocot seed

- Seed coat
- Endosperm
- Embryonic leaves
- Cotyledon
- Primary root
- Embryo
Location of seed borne pathogen

Fungi, bacteria, TMV

BCMV, CMV, fungi, bacteria

Loose smut, BCMV
Factor affecting seed transmission

- Virus: different viruses have variable pattern
- Virus strains
- Host plant and variety: e.g. BCMV-90% in different varieties, BSMV: upto 75%
- Time of infection
- Location of seed on plant
- Age of seed
- Temperature
- Host resistance