Meristem Culture

For production of virus free plants

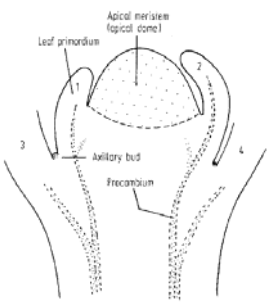
Meristem tip culture

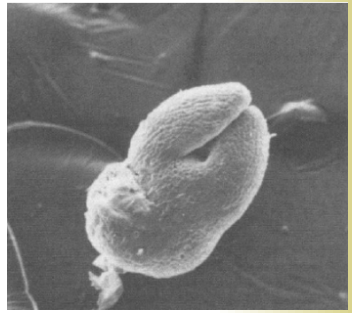
Apical meristem tips (domes of actively dividing cells with 1-2 leaf primordia) were excised in sterile conditions either from *in vivo or in vitro plants or highly proliferating* meristems

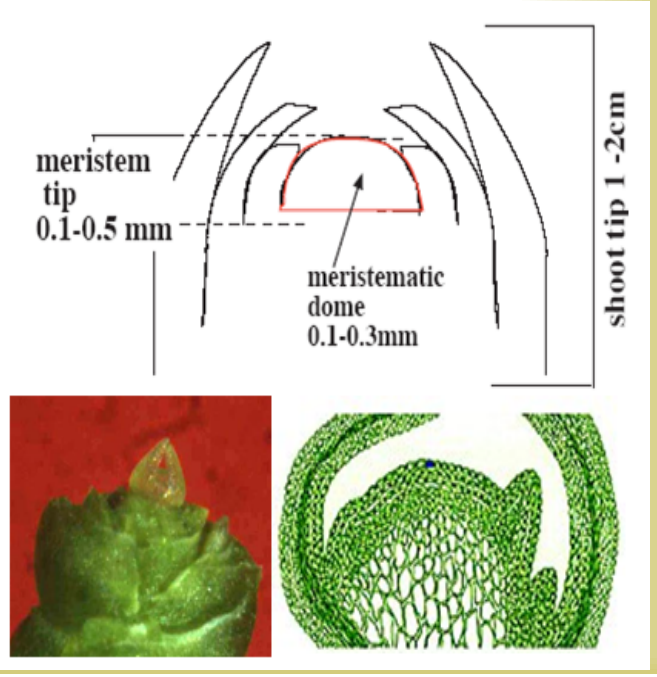
Transferred to glass tubes on 10 ml of solid MS medium

Tubes were maintained at 24 ± 1°C in dark conditions for 3 days, and then under standard illuminated conditions

Plantlets derived from meristem-tip culture usually retain the genetic characteristics of mother plant



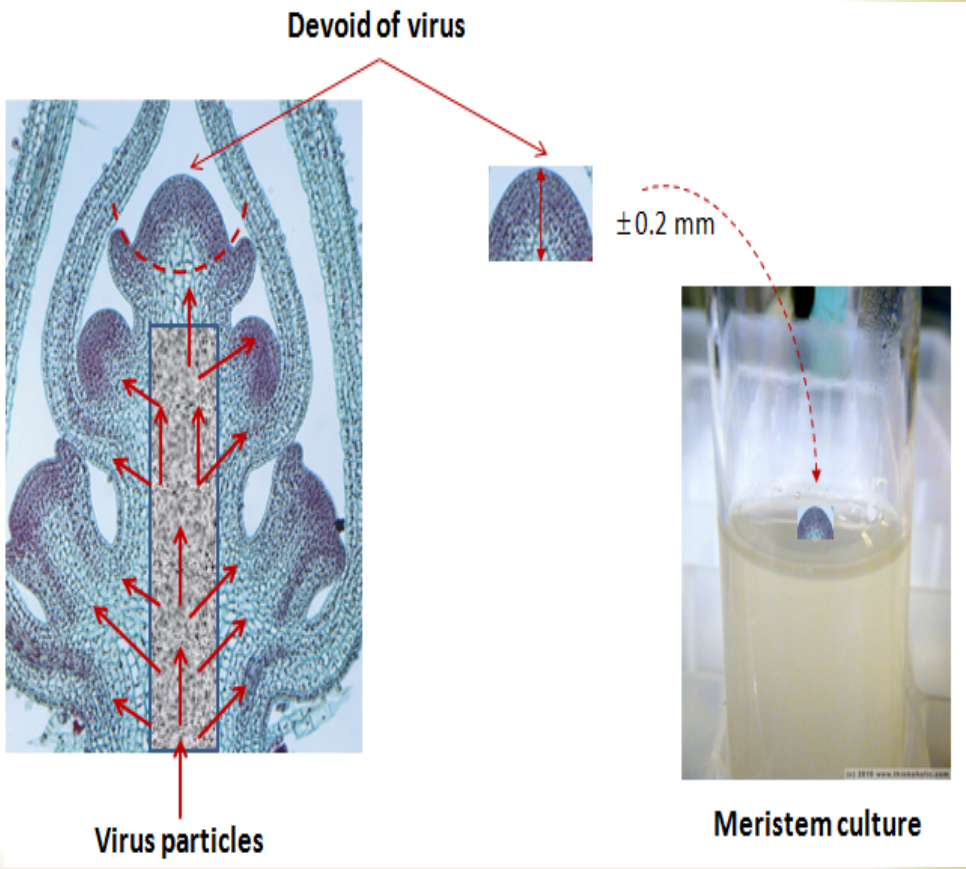




Mersitem culture - Why meristems are virus free ?

Failure to invade meristem is due to:

1. **Lack of a vascular system**. Spreading cell to cell via plasmodesmata which are too small to allow the passage of virus particles
2. **High metabolic activity**: active mitosis – the synthesis of RNA for viral multiplication may suppressed. Active metabolic process which is not suitable for virus multiplication
3. **High auxin concentration** in meristematic cells in hibit virus multiplication : interfere nucleic acid metabolism
4. **Competition** for nutrients enzymes for virus replication



What are viruses ?

- very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core
- carry genetic information encoded in their nucleic acid
- Translation of the genome or transcription and replication takes place within the host cell and uses some of the host's biochemical "machinery"
- Viruses do not store free energy and are not functionally active outside their host. They are, therefore, parasites (and usually pathogens)

How can viruses enter plant cells to cause a primary infection ?

- a purely **mechanical** injury that breaches the cell wall and **transiently breaches the plasma membrane** of underlying cells;
- similar **gross injury** due to the mouthparts of a **herbivorous arthropod**, such as a beetle;
- **injection** directly into cells through the **piercing mouthparts of sap-sucking insects or nematodes**;
- **carriage into** plant tissue on or in association with cells of a **fungal parasite**;
- **vertical transmission through infected seed** or by vegetative propagation;
- transmission via **pollen**; and
- **grafting** of infected tissue onto healthy tissue.

Why are viruses important?

- Cause many important plant diseases.
- Are responsible for huge losses in crop production and quality in all parts of the world.
- Virus is restricted to certain parts of the plant (e.g. the vascular system; discrete spots on the leaf) or spreads throughout the plant causing a systemic infection.
- Infection does not always result in visible symptoms

- Infected plants may show a range of symptoms :
  - leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches),
  - leaf distortion (e.g. curling)
  - and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation).

Creating Virus-free Clones of Plants

- Thermotherapy : 35-40°C. Any problems ??
- chemotherapy : azaguanin, thiouracyl, zinc sulphate, malachite green, etc.
- **Tissue (meristem) culture methods** - micrografting

have been used either alone or in combination to eliminate viruses

Heating tretament

- Inactivation of intact virus particles by breakage of their RNA.
- Disruption of virus particle with enzymatic degradation of its components
- Inactivation of accessory enzyme: virus RNA polymerase lead to shutdown viral RNA synthesis.
- Prevention of virus particle assemblies : coat protein can not assume the configuration

Host	Virus eliminated	Temperature
Chrysanthemun	Chrysanthemum B virus	35 – 38 °C
Carnation	Carnation ringspot virus 35 to 40oC Carnation vein mottle Viric virusus	35 – 40 °C
Banana	Cucumber mosa	35 – 43 °C
Goose Berry	Gooseberry vein banding virus	35 °C
Potato	Potato virus Y,S,X	35 – 38 °C

Chemotherapy

- The use of chemicals to suppress virus symptoms and multiplication in infected plants
- Use of antiviral compounds-Ribavirin/Virazole, DTH
- Growth promoting chemicals- cytokinins
- Antimetabolite chemicals- Azaguanine, Thiouracil
- Actinomycin D and cycloheximide B

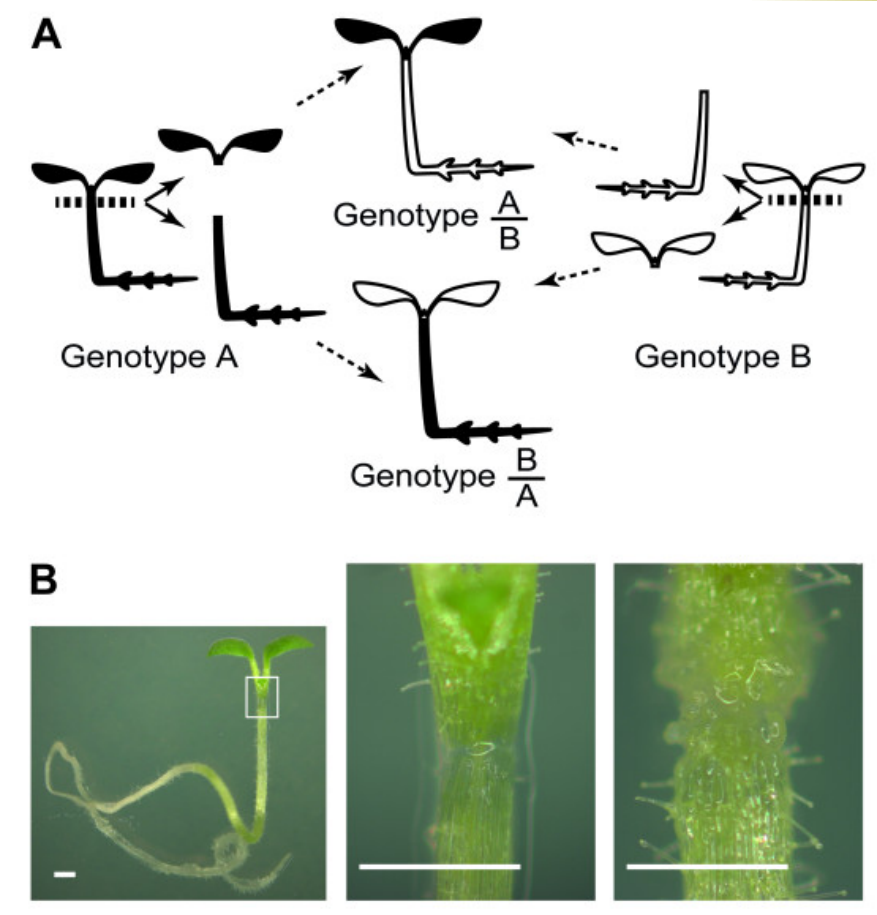
Factors influencing Virus elimination

Virus elimination depends on:

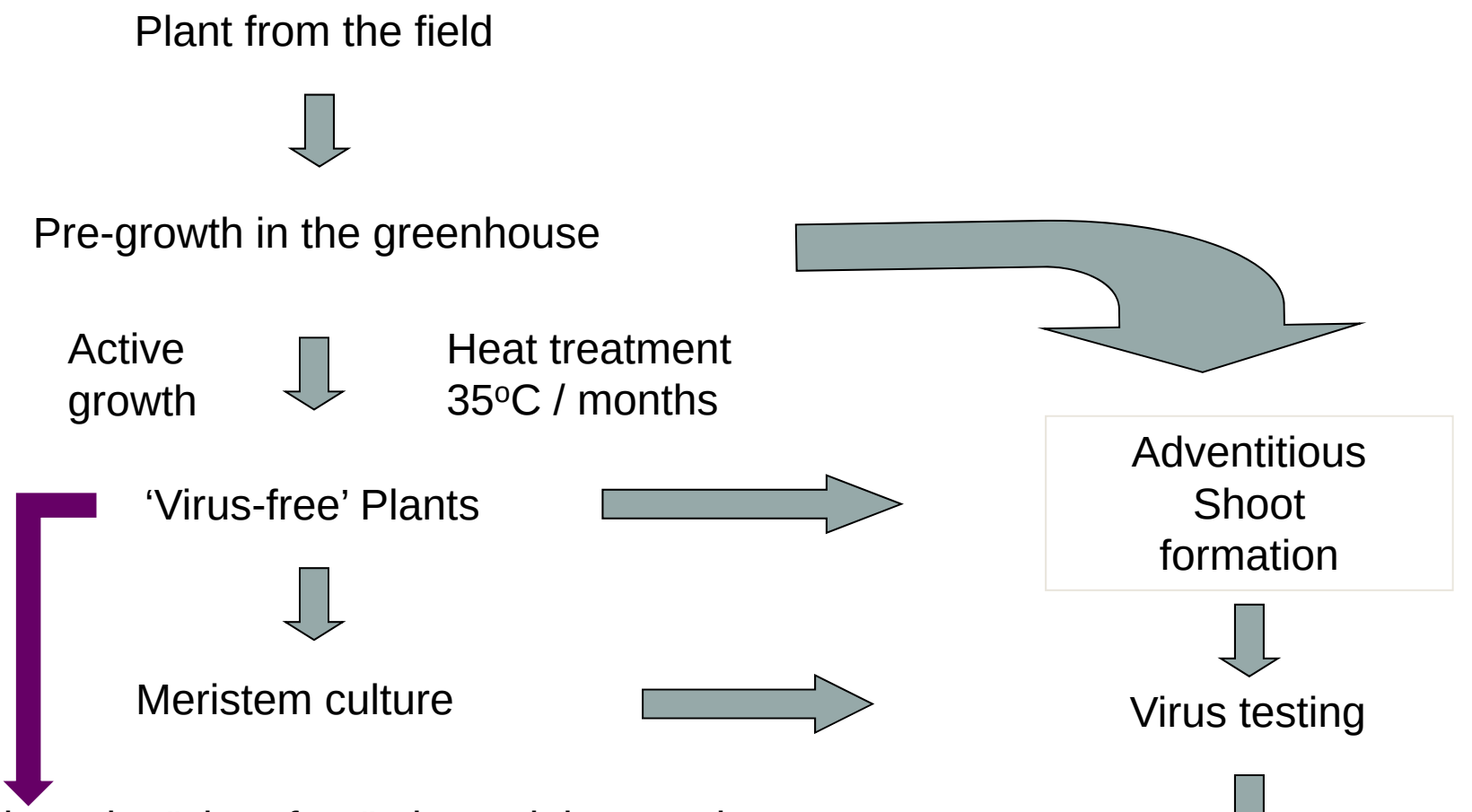
- Meristem explant size
- Bud location : terminal bud >have stronger growth potential than lateral.
- Season: early spring & early autumn > winter & summer; after dormancy of storage organs
- Heat treatment : in water or in air
- Culture media : high concentrations of growth hormones inhibit virus growth

Micrografting

- Meritem tip can be grafted onto a rootstock
- Meristem (0.1 to 0.4mm) excised from the infected cultivars
- Aseptically grafted onto the vascular ring of a decapitated virus free rootstock
- Culture of grafted plantlets in vitro
- Transfer plants to soil and maintain
- Suitable for woody species (fruit crops)
- Indexing for viroid, viruses, and phytoplasma



Elimination of viruses



Since the "virus-free" plant might contain a virus you did not know about, it is proper to call them
virus-tested or virus indexed plants.

Virus Indexing

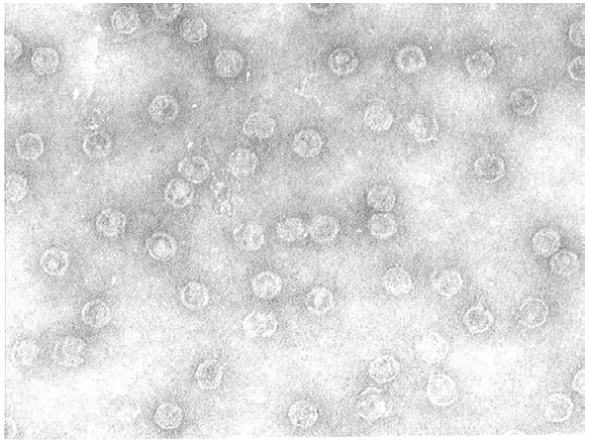
- **Indexing** means detecting virus : by physically transmitting it from infected host plant to a sensitive **indicator plant**

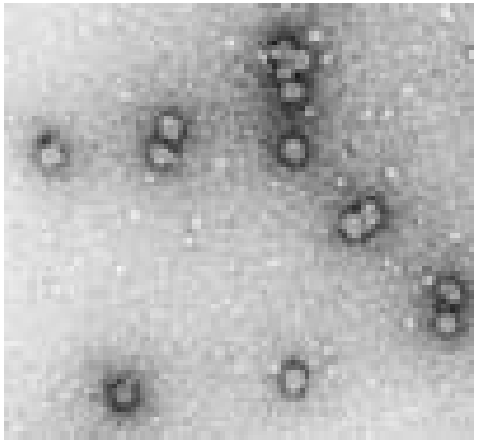
- Ex. : grafting a leaf from a test plant onto an indicator plant
  Visual symptoms : any virus is present in the plant tested or not

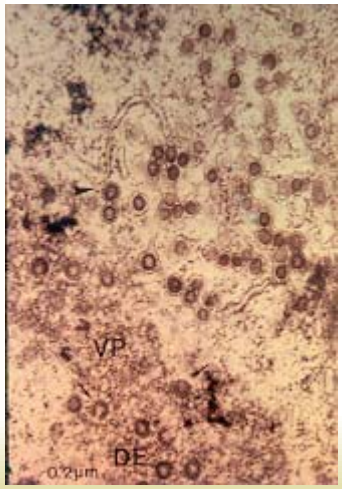
Virus indexing – Biological assays

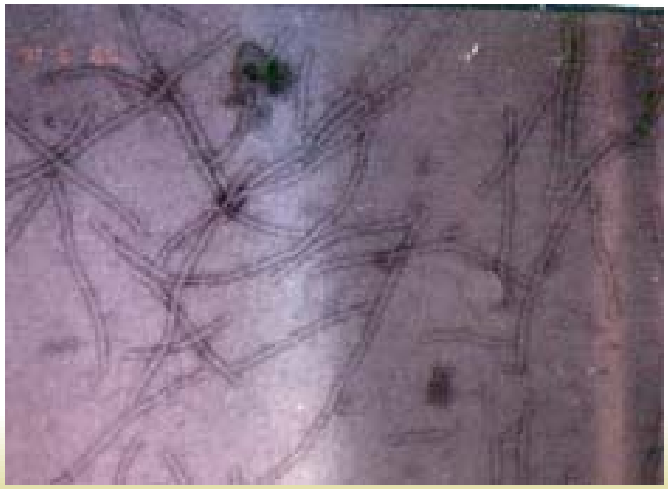
- Indicator hosts
- Transmission by graft inoculation

Virus indexing – Physical assays









Virus indexing – Serological assays

- Immuno Electron microscopy
- Enzyme linked immunosorbent assay (ELISA): an enzymatic color reaction indicates that a virus is present in the test plant.
- Dot immunobinding assay (DIBA)
- Tissue blot immuno assay (TIBA)