Production of virus Free Plants

- Tissue culture is an excellent tool for multiplying, maintaining, storing and distributing plants.
- Maintaining unique plants for breeding, propagation, or distribution can be expensive in terms of the time, space and labor required.
- In-vitro propagation of apical meristems is an important part of virus-elimination therapy for improving the health of plant collections.
- Distribution of tissue cultured germplasm often assists breeders and nurseries in meeting quarantine regulations.

Production of virus Free Plants

- **Germplasm may be a source of genes for improving such traits as insect and disease resistance, improved quality, drought or cold tolerance, increased yields, low chilling requirements, adaptation to mechanical harvesting or longer shelf life in distribution and retail channels.**
- **Foreign pathogens continue to pose a considerable threat to crops. Prevent introduction of quarantine pathogens in imported prohibited germplasm using a range of diagnostic techniques to intercept them**
- **Develop improved methods of detecting quarantine pathogens and investigate the etiology of poorly described diseases and pathogens of quarantine significance**
- **Eliminate quarantine pathogens from valuable plant germplasm**
- **Complete therapy on sweet potatoes infected with geminiviruses.**
- **Provide tissues from in vitro therapy for PCR detection of geminiviruses in sweet potatoes**

WHY DEVELOP CERTIFICATION AND INDEXING PROGRAM

- To stop the distribution of viruses in propagative plant material
- To avoid losses due to viruses
- An insurance against distribution of exotic and destructive viruses
- To lower the inoculum potential of viruses with in the country
- To determine the type of viruses present in a state or country

CERTIFICATION AND INDEXING PROGRAM DEPENDS ON

- Proper identification and detection of virus
- Development of sensitive and reliable indexing method(s)
- Proper selection of plant material and detection method
- Knowledge of host, pathogen and environment
- Maintenance and multiplication of virus free material
- Distribution and Propagation of pathogen free material under high hygiene

Meristem tip culture

- Apical meristem tips (domes 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 in a growth cabinet (culture room) at 24 ± 1°C in dark conditions for 3 days, and then under standard illuminated conditions

Mechanism of virus Elimination in meristems

- Failure to invade meristem is due to:

1. High auxin concentration in meristematic cells
2. Competition for nutrients enzymes for virus replication
3. Active metabolic process which is not suitable for virus multiplication
4. Action of growth regulators (Cytokinins)
5. Presence of inhibitors (Phenolamines)
6. Metabolic disruption of enzymes necessary for viral replication, RNA degradation

Characteristics of meristem tissues

- Apical meristem-during embryo development, is a dome of actively dividing cells located at the apex of shoots and roots.
- Plantlets derived from meristem-tip culture usually retain the genetic characteristics of mother plants
- Virus elimination from selected plants-do not differ phenotypically from mother plants
- Variants comparable to traditional methods of propagation
- Genetic stability of the germ line is prerequisite
- Genetic stability of meristems under the strict control of DNA synthesis.
- Avoids Spontaneous chromosomal structural changes
- Undifferentiated tissues such as apical meristems uniformity of diploid state of cells is maintained

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

Virus elimination through heat treatment

Preparation for therapy: **surface sterilization & culture establishment**

Temperature treatment: **Three weeks after subculture *in vitro* shoots are transferred to a special growth chamber and grown at an increased temperature regime for 14 to 21 days**

Regeneration and indexing: **Regeneration of small plantlets occurs within a few weeks. After indexing virus free plants selected and multiplied further**

Acclimatization: **Plantlets are rooted *in vitro* and transferred to the greenhouse. After careful hardening potted plants are re-tested for their pathogen-free status**

Multiplication of Virus Free Plants:

For budwood production plants are transferred to an insect proof screenhouse.

Thermotherapy

• High temperature treatment has been widely used in production of virus free plants (30 to 40^{o}C)

Host	Virus eliminated	Temp.
Chrysanthemum	Chrysanthemum B virus	35 to 38^{o}C
Carnation	Carnation ringspot virus Carnation vein mottle virus	35 to 40^{o}C
Banana	Cucumber mosaic virus	35 to 43^{o}C
Goose berry	Gooseberry vein banding virus	35^{o}C
Potato	Potato virus Y, S, X	33 to 38^{o}C

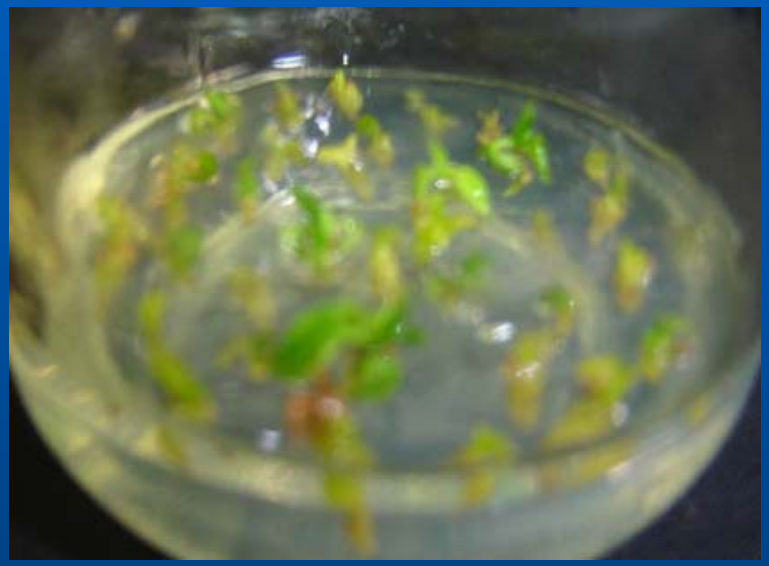
Chemotherapy

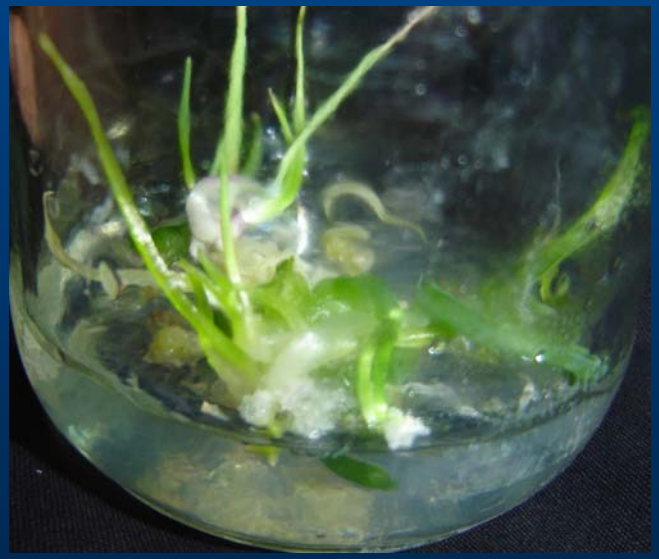
- The use of chemicals to suppress virus symptoms and multiplication in infected plants
- Use of antiviral compounds- Ribavirin/Virazole, DTH
- Growth promoting chemicals-cytokinins
- Antimetabolite chemicals-Azaguanine, Thiouracil

Electrotherapy

- The application of electrical pulses to eliminate viruses from plant tissue has recently received much attention.
- Quacquerelli et al. [1980] obtained symptomless almond plants and Lozoya-Saldana et al. [1996] reported on the elimination of PVX from different clones of potato.
- Using an electrotherapy apparatus developed in Cuba (Patent Cuba 37/95 AO 1C/08 1524/97),
- Hernandez et al. [1995] treated garlic (*Allium sativum* L), sugar cane (*Saccharum* sp. *hibrido* L.), potatoes (*Solanum tuberosum* L.) and araceas (*Xanthosomas* and *Colocasia*) for Potyvirus, Luteovirus and Carlavirus elimination respectively.
- For banana (cv. W. Bungulan (AAA)), Hernandez et al. [1996] reported BSV elimination in approximately 40-80% of regenerated plants.

Virus elimination in onion-Chemotherapy





Virus Elimination

- Virus elimination depends on:
- Meristem size
- Type of virus/Phytoplasma
- Virus strain
- Plant species
- Cultivar type
- Physiological condition of mother plants and position of meristem

Virus indexing

- **Biological assays:**

  A. *Indicator hosts*

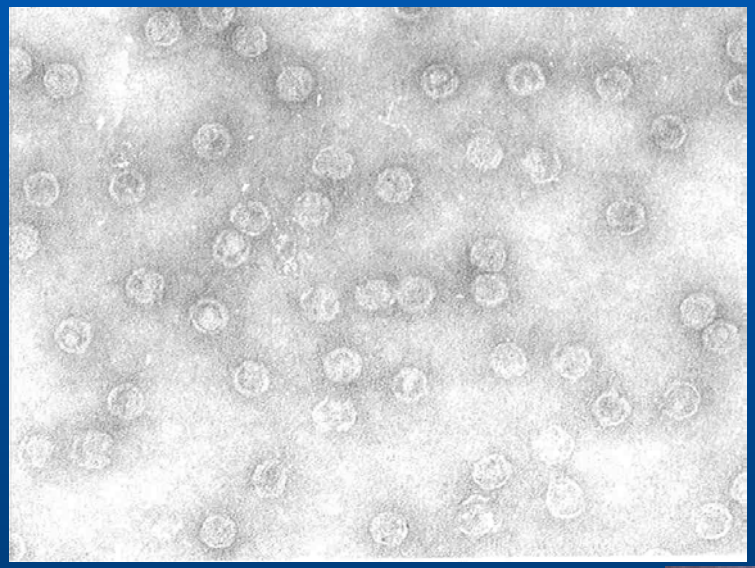
  B. *Transmission by graft inoculation*

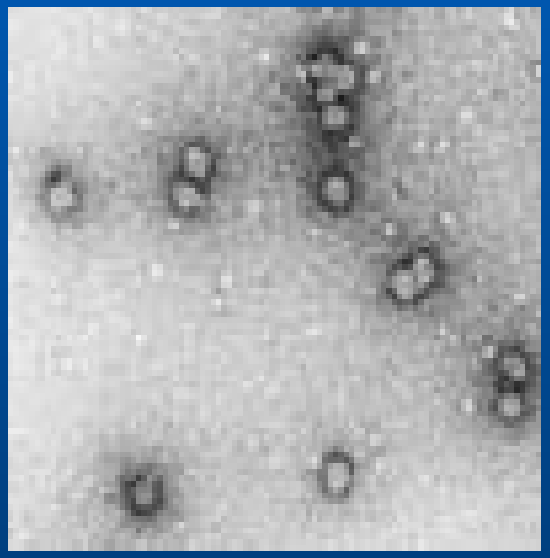
  C. *Transmission by Dodder*

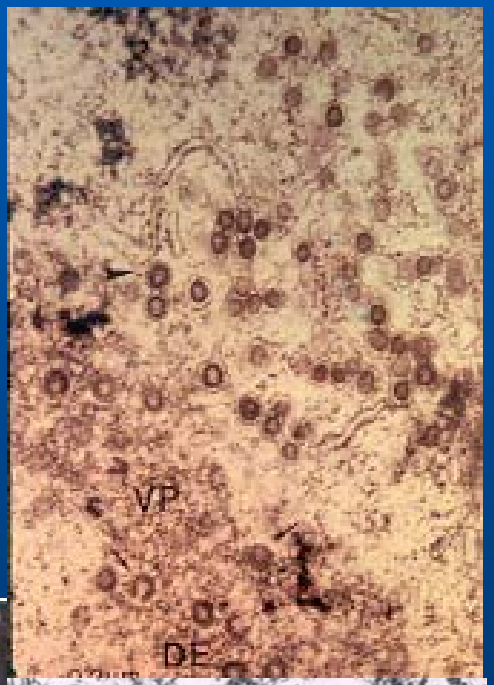
  D. Vector transmission

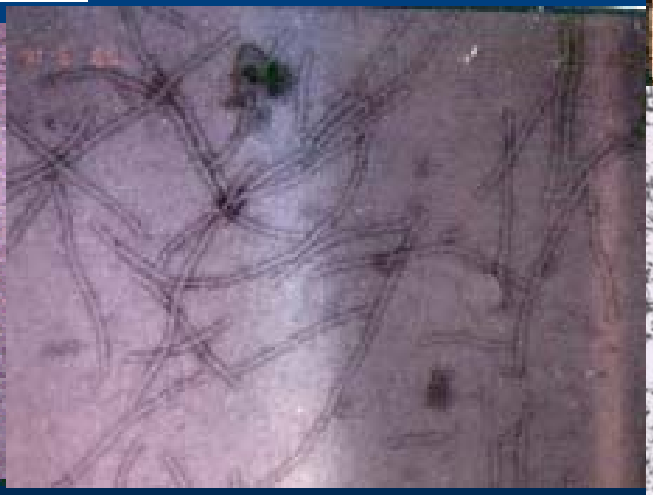
Virus indexing

Physical assays: A. *Electron microscope*









Virus indexing

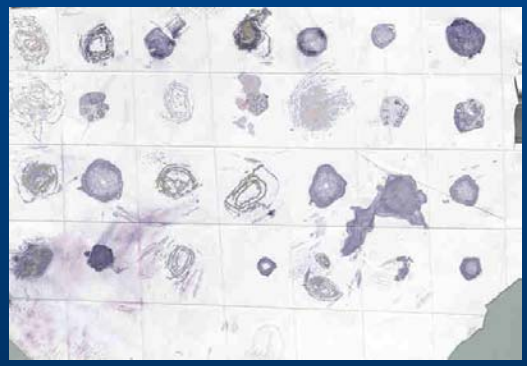
- **Serological assays:**

  A. *Immuno Electron microscopy*

  B. *Enzyme linked immunosorbent assay* (ELISA)

  C. *Dot immunobinding assay (DIBA)*

  D. Tissue blot immuno assay (TIBA)



Virus indexing

- **Molecular assays:**

  A. *Double stranded RNA (dsRNA) an*

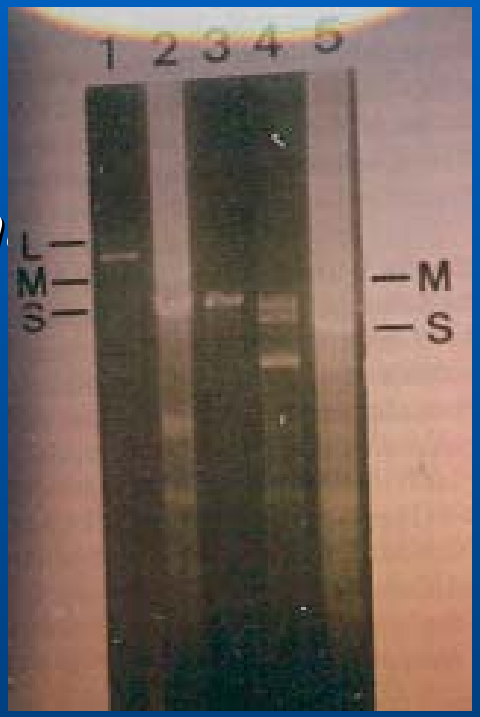
  B. *Molecular hybridization analysis*:

  1. Dot blot assay/ Squash blot
  2. Southern blot assay
  3. Northern blot assay

  C. *Microarrays*

  D. Polymerase Chain reaction (PCR)

  1. Multiplex PCR
  2. Immunocapture PCR (IC-PCR)
  3. Nested PCR
  4.Competitive fluorescence PCR (CF-PCR)



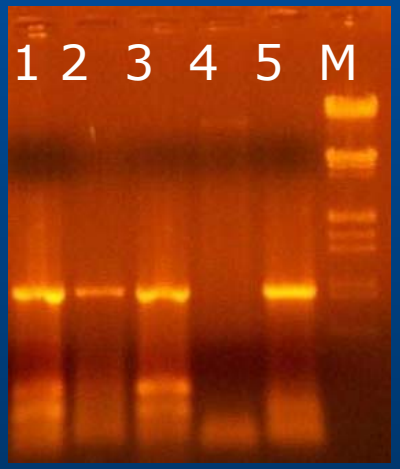
Development of Membrane based nucleic acid Protocol for PCR detection of BBTV

1. Blotting of tissue on membrane

2. Dissolving of membrane or mixing with PCR reaction mixture

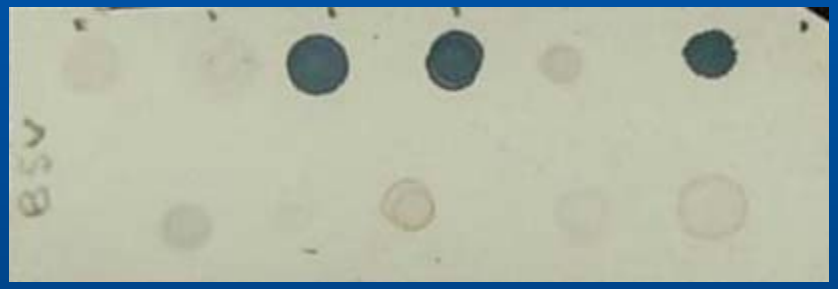
3. PCR amplification

4. Visualization of bands

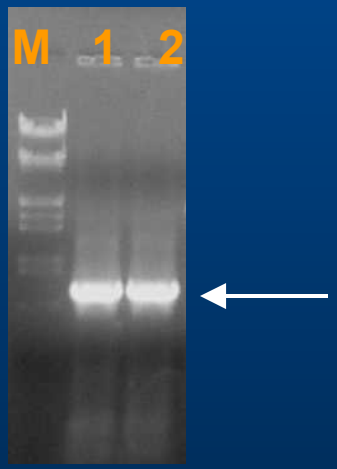


PCR detection

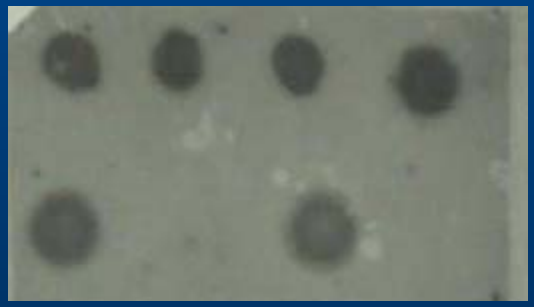
Development of nucleic acid probes for the Diagnosis of Banana Streak Virus



Colorimetric detection by dot hybridization

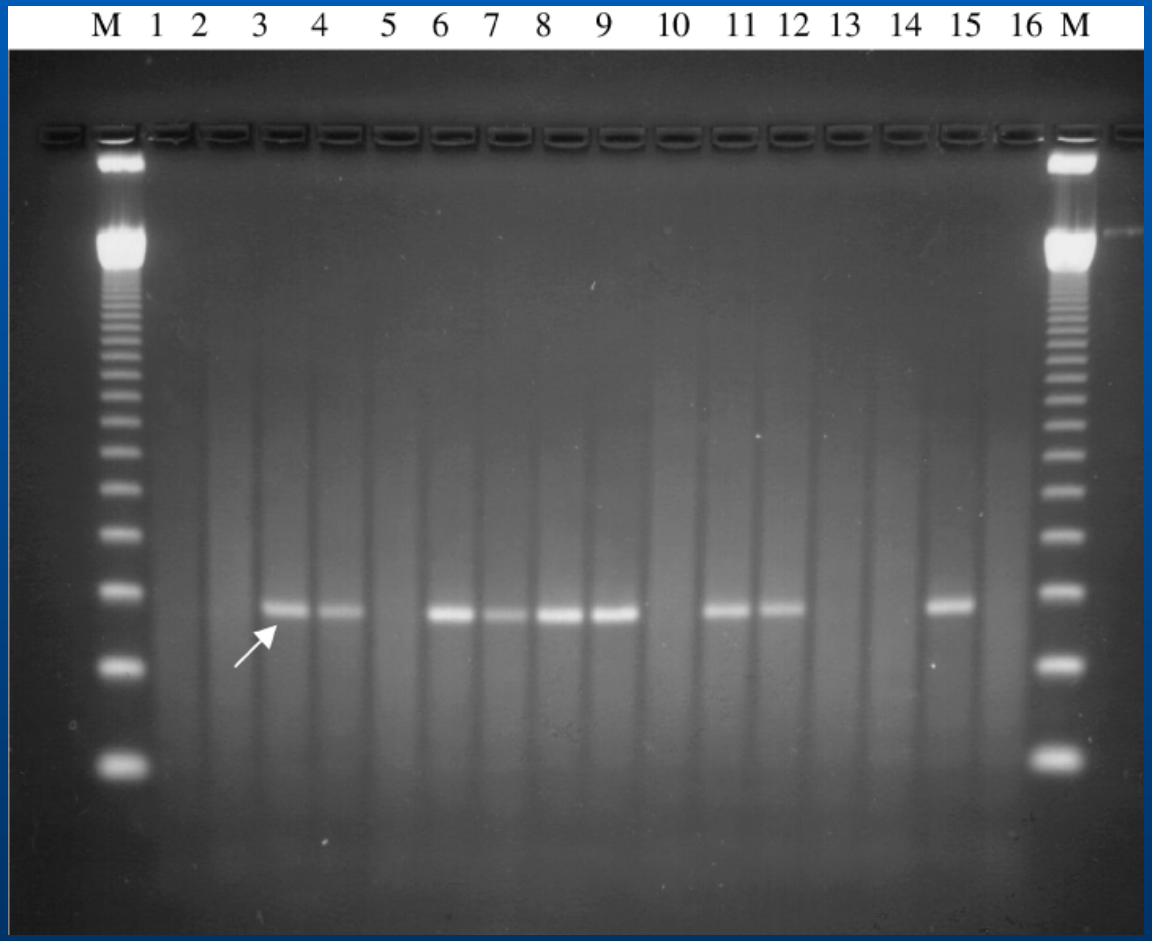


PCR Amplification and labeling of DNA

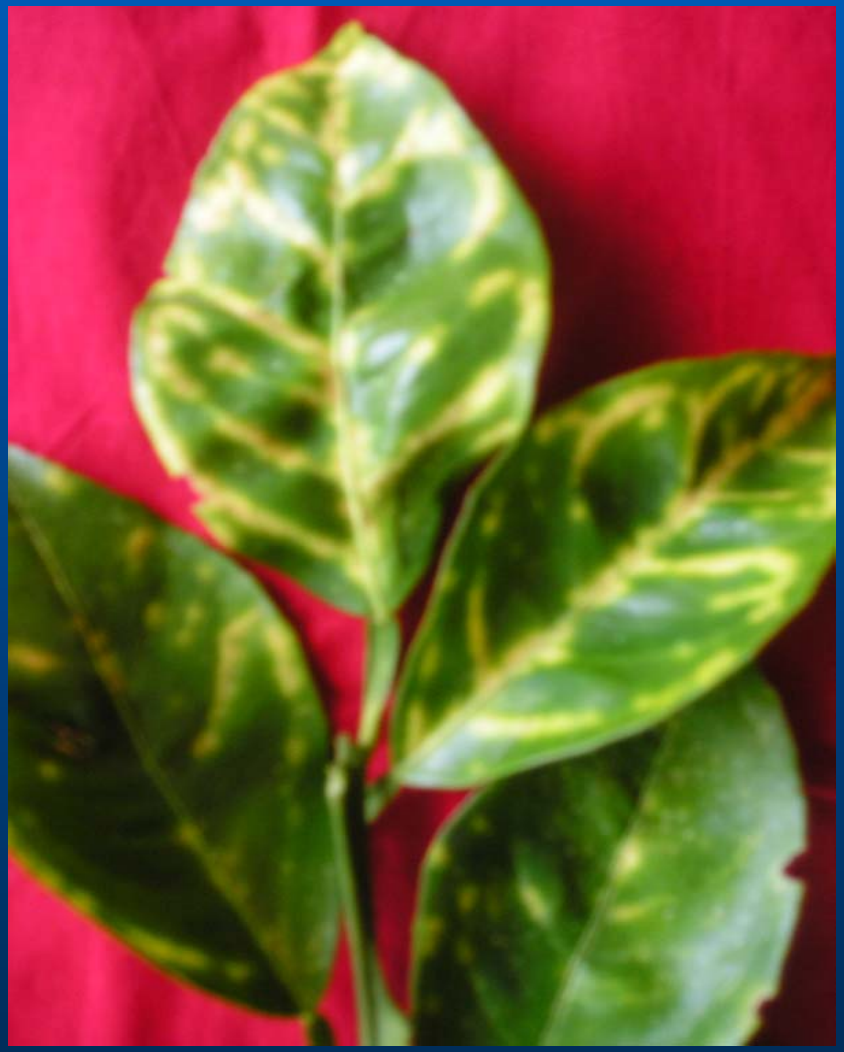


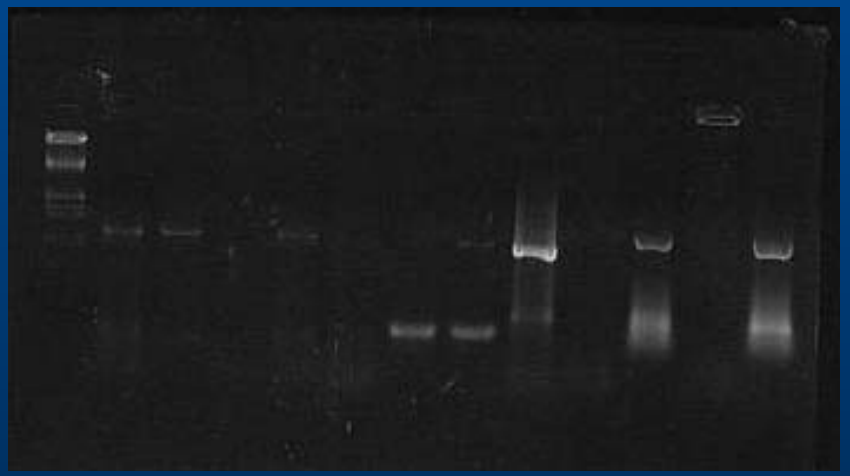
Chemiluminesnce detection by Dot Blot Assay

Virus indexing by PCR

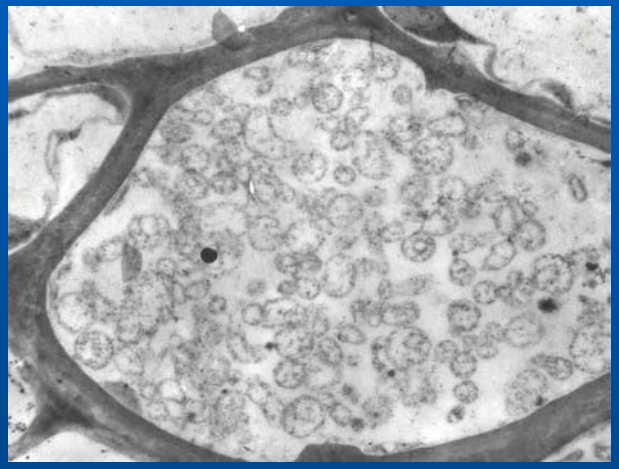


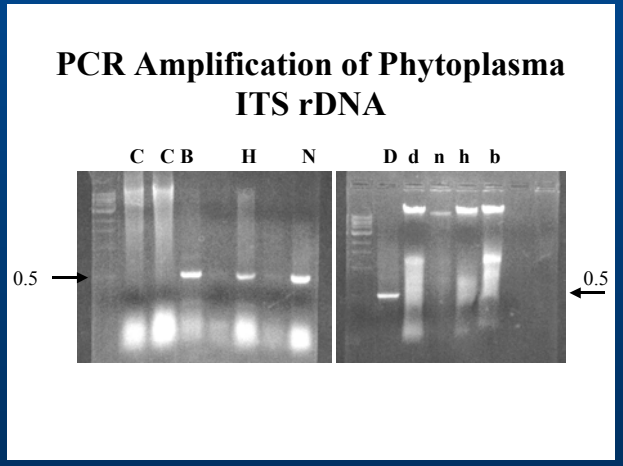
Citrus Yellow corky vein Viroid



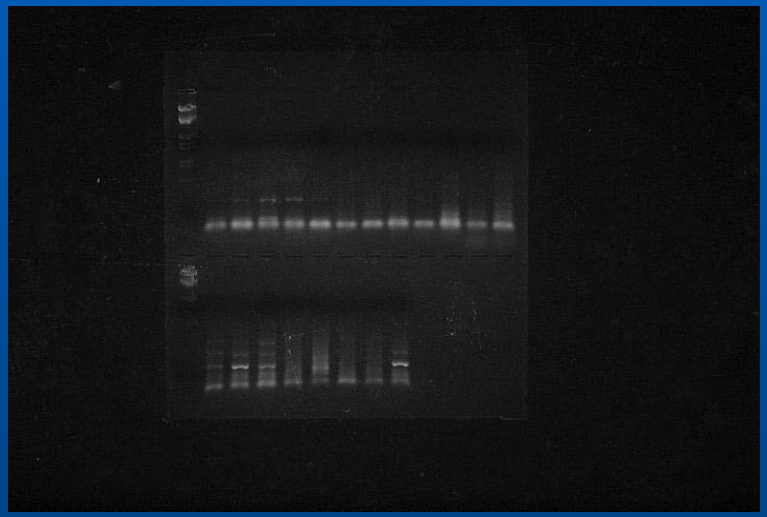


Diagnosis of Citrus Witches Broom





Tissue culture indexing



Detection of Viruses in Tissue Culture Banana From M/S Cadilla, Ahamdabad

Crop	Virus	No Tested	Detection of Samples by			
			EM	ELISA	NAP	PCR
Banana	BBTV	50	NT	NT	12	12
	BBrMV	56	0	22	NT	12
	BSV	50	NT	NT	0	1
	CMV	53	0	2	NT	2
	Unknown	10	3	3	NT	NT
	Total	209	3	27	12	27

Detection of Viruses in TC Plants from Shri Ramco Biotech, Bangalore

Crop	Viruses	No Tested	Method of Testing by			
			EM	ELISA	NAP	PCR
Banana	BBTV	70	NT	NT	4	8
	BBrMV	115	6	4	NT	2
	BSV	70	4	NT	0	8
	CMV	70	8	12	NT	8
	Unknown	20	10	6	NT	NT
Caladium	DsMV	12	10	4	NT	NT
	Poyvirus	12	10	6	NT	4
	INSV	12	NT	0	NT	NT
	Total	381	48	32	4	30

Anticipated products of Practical utility expected to be evolved

- Polyclonal antibodies against the purifiable viruses infecting tissue culture plants in India
- Monoclonal antibodies against selected viruses
- cDNA probes for detecting viruses and virus-like pathogens which are not amenable to serodiagnosis, like the geminiviruses, ilarviruses, viroids and phytoplasmas.
- Diagnostic kits for the most commonly occurring viruses.
- Quality markers for specific species
- Database of the DNA fingerprints of the target germplasm.
- Tested virus-free mother plants.
- Tested virus-free and true to type tissue culture plants for distribution.

Web Site: Online Registration

www.dbtqtestplants.org

A web site has been launched which gives details of the services provided. There is online registration for all Industries / Users who wish to avail of this facility for certification of their products.

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NATIONAL FACILITY FOR VIRUS DIAGNOSIS AND QUALITY CONTROL

IIHR

सत्यमेव जयते

Department of Biotechnology
Ministry of Science & Technology
Government of India

Plants identified for virus and quality testing

Fruit Crops:

Banana; Papaya; Strawberry

Spice Crops

Black pepper; Clove; Large cardamom; Small cardamom; Vanilla

Ornamentals

Aglaonema; Aistomeria; Alocasia; Anthurium; Asparagus; Begonia; Caladium; Calathia; Carnation; Chrysanthemum; Colocasia; Diffenbachia; Ficus; Fittonia; Freesia; Gerbera; Gladiolus; Iris; Kalanchoe; Lily (Asiatic, Oriental, Long folium); Limonium; Orchids; Phalaenopsis; Philodendron; Rose; Spathiphyllum; Syngonium; Xanthosoma; Zantedeschia

Forest Trees

Bamboo; Eucalyptus; Poplar; Teak