Virus Diagnostic and Planting Material Production

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Viral disease

First report: Holliday (1959)

India: From Idukki during 1975 (Paily et al. 1981)

Also known as mosaic disease (India)

Wrinkled leaf disease (Malaysia)

Stunted disease (Indonesia)

Little leaf (Sri Lanka)

Occurrence

- Brazil, India, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand, Vietnam
- Yield loss vary depending on time of infection
- Yield declines gradually
- Yield loss vary from negligible to upto 80%

Incidence of the disease in Karnataka and Kerala, India

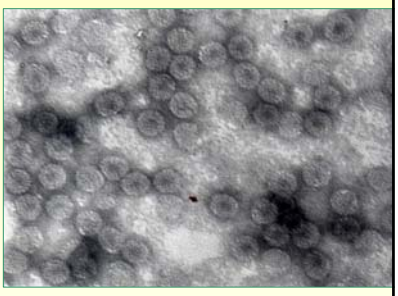
State /District	Range of incidence (%)	Mean incidence (%)
Karnataka Dakshina Kannada	0	0
Hassan	0-20	5.2
Madikeri	0-78	14.9
Uttara Kannada	0-2	0.4
Kerala Idukki	0-78	29.4
Kannur	2-42	19.5
Kasargod	0-53	18.9
Kozhikode	0-33	10.7
Wynad	13-83	45.4

Disease symptoms

- **Mosaic, mottling and small leaf conditions**
- **Symptoms are prominent on young & emerging leaves especially during April-May months (Indian conditions)**
- **Some of the affected plants may not show any visible symptoms during certain months**
- **Appearance of symptoms are more in neglected, poorly nourished and old gardens**

- **Vein clearing, crinkling, chlorotic mottling, curling, brittle, leathery and chlorotic patches/streaks on leaves**
- **Narrow leaves with reduced internodal length leading to stunting of plants**
- **Reduced vigor and yield**
  Early infected plants are severely stunted with low yield

Two viruses are involved



1. *Cucumber mosaic virus (CMV)*

Isometric virus wtih ssRNA genome

Cause mosaic and stunting symptoms

Spreads when infected material is used for planting

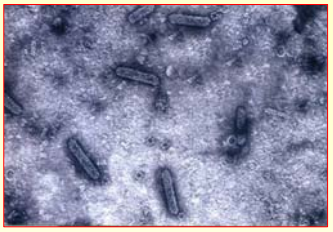
CMV infects many crop and weed hosts

Disease can be transmitted through grafting

Aphids can transmit the disease from diseased to healthy plants

Piper yellow mottle virus (PYMoV)

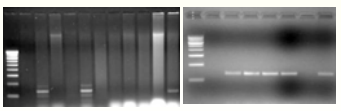
Bullet shaped virus with dsDNA as genome



Cause yellow mottling along veins and curling

Spreads through infected planting material

Transmitted by mealybugs (*Ferrisia virgata, Planococcus citri, P. elisae*), black pepper lace bug (*Diconocoris distanti*)

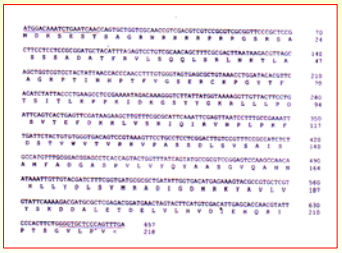


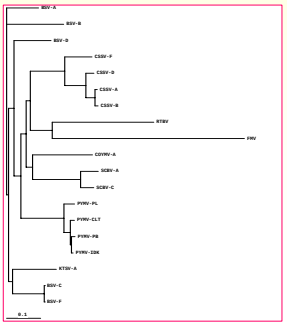
Through seeds

Transmission through mealybugs

P. Citri ***F. virgata***

Cloning and sequencing of CMV infecting black pepper showed that it belongs of subgroup IB





Cloning and sequencing of portion of ORF I and III of PYMoV revealed conserved ORF III region among badnaviruses while ORF I was variable

Viruses are systemic in nature and can not be killed by chemicals

As black pepper is vegetatively propagated primary spread of virus occur through use of stem cuttings from infected plant

Hence identification of virus-free plants for propagation is important

As masking of symptoms are seen in infected plants, symptoms can not be a reliable method for identifying healthy plants

Use of sensitive methods such as ELISA and PCR are necessary to identify virus-free plants

ELISA based methods failed to provide fool-proof detection of PYMoV owing to low titre of the virus in plants

Hence a PCR based method is necessary for indexing to identify virus-free black pepper plants

- **Detection of CMV by RT-PCR**
- **Detection of PYMoV by PCR**
- **Combined detection of CMV and PYMoV by mRT-PCR**

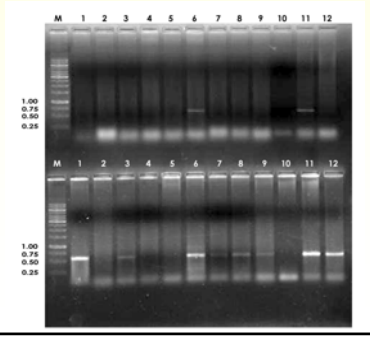
Detection of CMV by RT-PCR

Isolation of total RNA from test samples

Perform RT-PCR using primers specific for CMV.

A known positive (CMV infected) and negative (healthy black pepper) controls were used with each set of RT-PCR

After RT-PCR, contents were run on the gel and plants were scored as positive or negative based on the presence or absence of specific bands.



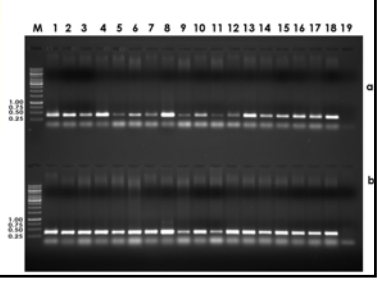
Detection of PYMoV by PCR

Isolation of total DNA from test samples

Perform PCR using primers specific for PYMoV

A known positive (PYMoV infected) and negative (healthy black pepper) controls were used with each set of PCR

After PCR, contents were run on the gel and plants were scored as positive or negative based on the presence or absence of specific bands.



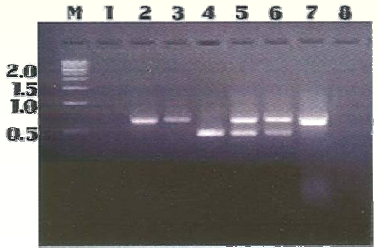
Multiplex RT-PCR for detection of PYMoV and CMV

Isolation of total nucleic acid (both RNA and DNA)

Perform mRT-PCR using primers for PYMoV and CMV

A known positive (PYMoV and CMV infected) and negative (healthy black pepper) controls were used with mRT-PCR

After mRT-PCR, contents were run on the gel and plants were scored as positive or negative based on the presence or absence of specific bands.



Planting material production

Only plants tested as virus-free by PCR should be used as source of mother plant for further propagation

Establishment of Mother garden

Good bearing and disease free vines of known variety should be indexed for viruses

Only virus-free plants from them should be planted in mother garden

It is advisable to maintain these mother plants under insect-proof conditions

They should be periodically checked for viruses

Methods of propagation

Conventional method

Rapid multiplication
- **Bamboo method**
- **Mound method**

Serpentine method

Micropropagation

Rapid multiplication by Bamboo method

Serpentine method

Micropropgation

Procedure and standard parameters for production of Black pepper by tissue culture

*Selection of superior mother plant as explant source

Culture Initiation and establishment of nodal and shoot tip meristem → Virus free certification & DNA sampling

Multiplication for 2nd /3rd subcultures

Screening for systemic bacterial resistance/to antibiotic sensitivity

** Elimination of phenolic exudation

Bacteria & phenol-free mother cultures (4-5th subculture)

*** Multiplication of shoots (10 passages)

Elongation of shoots

In vitro rooting

Hardening inside the green house

Acclimatization in Nursery → Virus free & genetic fidelity certification vis-à-vis reference DNA

Dispatch

Soil borne diseases

Foot rot	*Phytophthora capsici*	Baiting, microscopy, ITS-PCR-RFLP, SCAR, Species specific primers
Slow decline	*Radopholus similis, Meloidogyne* spp.	Microscopy, rDNA-PCR

Survive in soil / planting material and are carried to the field inadvertently

In order to prevent chance contamination, the potting mixture should be heat sterilized using steam or by soil solarization

Soil solarization

Steam sterilization

Production of disease-free planting materials

Establishment of Mother garden

Good bearing and disease free vines of known variety indexed for viruses

Only cuttings from virus-free plants are planted in mother garden

It is advisable to maintain these mother plants under insect-proof conditions

They should be periodically (at least once in a year) indexed for viruses and other pathogens

Regular monitoring and rouging of diseased plants should be done

Whenever insects (aphids, mealybugs) are seen, spraying with insecticides is necessary

Multiplication of planting material in nurseries

Cuttings from bearing mother vines are raised in a nursery under insect-proof conditions

Potting mixture is sterilized (steam or soil solarization) and fortified with beneficial micro organisms such as *Trichoderma harzianum, Pseudomonas fluorescens*

Nursery plants also have to be checked for pathogens periodically (0.1-1% of plants, depending on the lot/ batch size of plants produced)

Regular monitoring and rouging of diseased plants should be done

Whenever insects (aphids, mealybugs) are seen, spraying with insecticides is necessary

The pathogen-free stocks from the nurseries are then multiplied in secondary nurseries or used for commercial planting.

Conclusions

Diseases are production constraints and reliable identification of pathogens is important

ELISA and PCR based diagnostics are developed for detection

Parameters for production of disease-free planting materials have been developed

If used properly, this would lead in the production of disease-free planting materials

Need to establish planting material production chain involving selection of parent material, initial testing and periodic testing of sub samples during multiplication and at the time of distribution of planting material

There is also a need for awareness creation on the importance of disease free planting material and capacity building of all stake holders

Future thrusts

Development of multiplex PCR/microarray for detection of all pathogens infecting black pepper

Development of easy to use diagnostic kits such as lateral flow device

Development of certification programme to produce disease-free planting materials

Thank You