Case study – Pepper mild mosaic virus (PMMoV), description of methods used for seed health test and result interpretation

Kai-Shu Ling

USDA-ARS, U. S. Vegetable Laboratory
Charleston, SC, USA

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U. S. Vegetable Laboratory

Broccoli

Collard green

Cowpea

Cabbage

Pepper

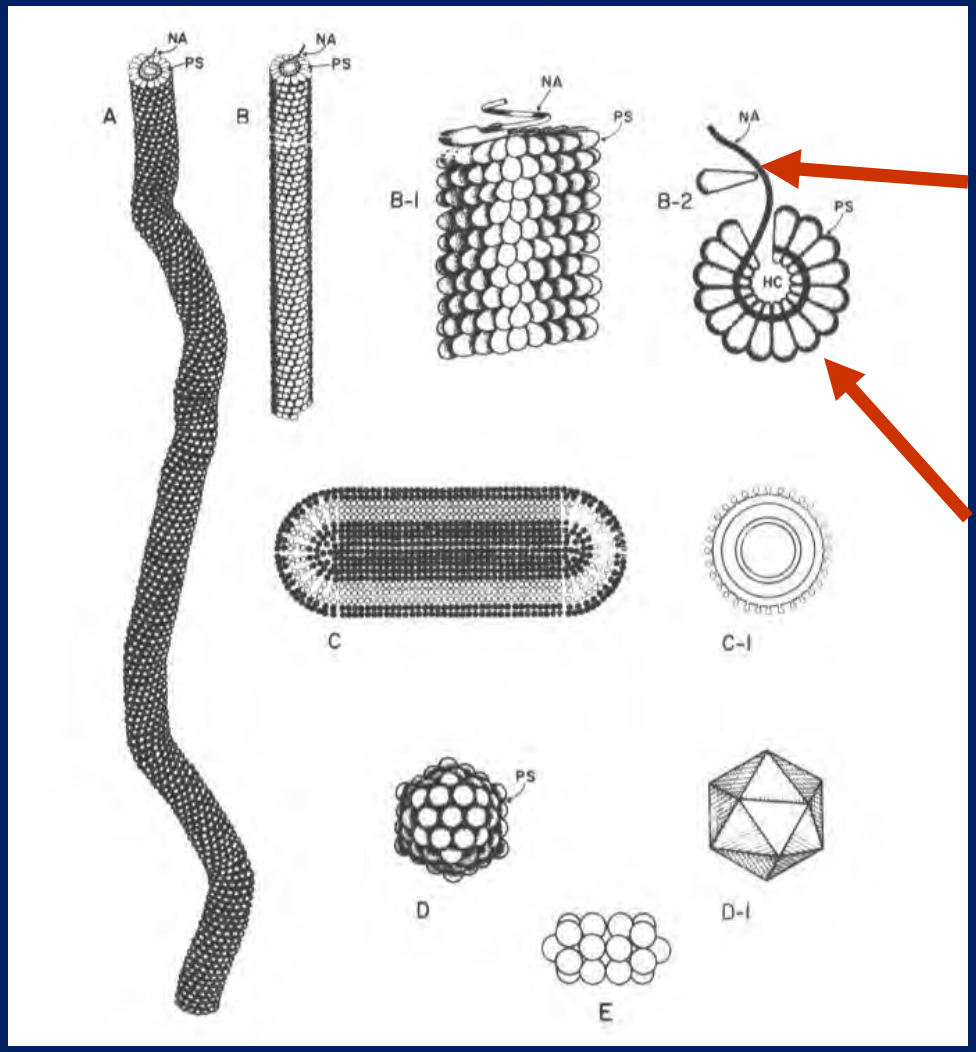
Sweetpotato

Cauliflower

Tomato

Watermelon

Virus structure, composition and the strategies in virus detection



Detection:

RNA or DNA → Molecular (PCR)

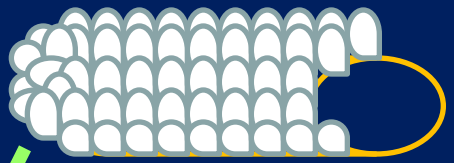
Coat protein → Serological (ELISA)

(From Agrios' Plant Pathology, 3rd ed.)

Common Methods for Plant Virus Detection

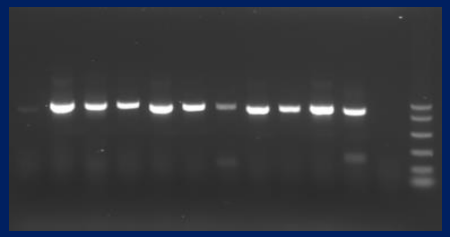
Indirect Tests (infectious + dead viruses)

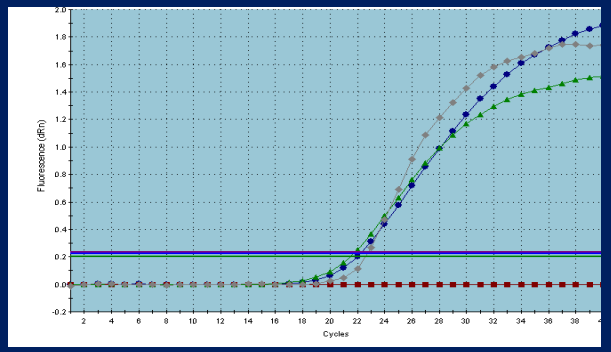
(quick, sensitive and specific, could result in false positive)



Coat protein

RNA or DNA





Direct Tests (infectious)

(slow, less sensitive, non-specific)

Major seed-borne viruses and viroids on Solanaceous crops and seed health tests

Genus	Species	Host	Indirect test		Direct test
			ELISA	qRT-PCR	Bioassay
Tobamovirus	PMMoV	Pepper	+	?	Local lesion on tobacco
	TMV	Pepper/ tomato	+		
	ToMV	Pepper/ tomato	+		
Potexvirus	PepMV	Tomato	+	+	Systemic on N. benthamiana
Pospiviroids	PSTVd/P CFVd	Pepper/ tomato	-	+	Growout

Pepper mild mottle virus

- Symptoms: leaf chlorosis (*mild*), plant stunting, and distorted fruits (*severe*).

- Spread:
  - Long distance: Seed-borne (*outer seed coat*).
  - Short distance: mechanical transmission (hands, tools, clothing). Particular in greenhouse production.
  - no insect vector. But in soil and water. Humans as a vector (stool and fecal pollution)?
- Distribution: Worldwide.

Pepper Seed-borne Tobamoviruses

- Family: Virgaviridae
- Genus: Tobamovirus
- Species:
  - Pepper mild mottle virus (PMMoV)
  - Tobacco mosaic virus (TMV)
  - Tomato mosaic virus (ToMV)

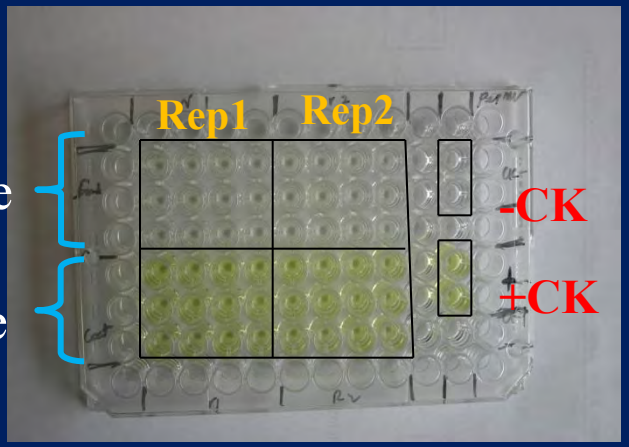
PMMoV-pepper seed

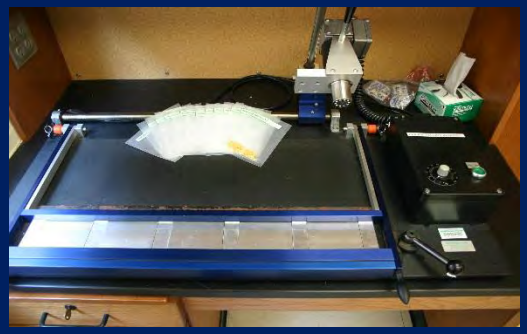
(PMMoV + TMV)

Seed health test by serological method (enzyme-linked immunosorbent assay -ELISA) for three tobamoviruses in pepper

ISTA/ISHI/NSHS: 3,000 seeds/lot in 250 seeds/subsample (12 subsamples in duplicate wells), tests for 3 viruses could be carried out using the same seed extract.

An absorbent value at OD405 nm that is 2x above the healthy control should be considered positive.





Bioassay with a local lesion test for tobamoviruses on tobacco plant

- ISTA/ISHI/NSHS: minimum 3,000 seeds in 250-500 seeds/subsamples (6-12 reps) through mechanical inoculation on tobacco, *Nicotiana tabacum* Xanthi NN to induce necrotic local lesions.
- When such sample is inoculated on to a healthy pepper plant, a new disease would likely occur.
- Local lesion test is quicker (5-7 days) than 4-5 weeks, quantification of virus titer by counting local lesions, local lesion is more obvious than mild mottle.

Risk assessment: Interpretation of seed health test results

- If following the standard method, a negative test result is used to certify a seed lot. Planting such certified seeds will likely not introduce that virus to a region or a country.
- Even if a seed-borne disease has established in a country, planting a certified virus-tested seed lot is still important to reduce initial virus inoculum, slow disease progression and minimize yield loss to an economic threshold level.

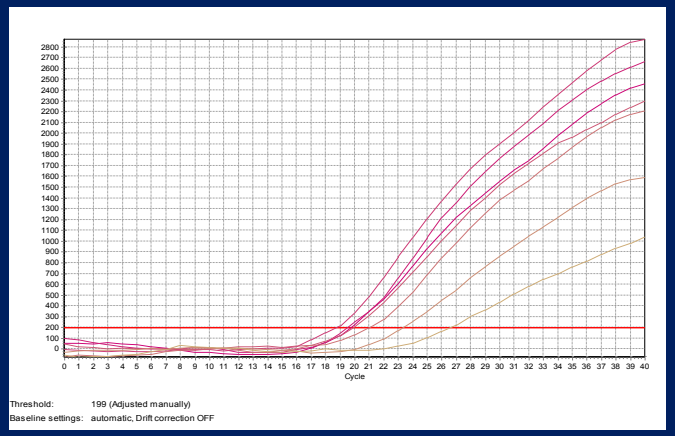
Risk assessment (cont.): Interpretation of seed health test results

- A right balance in seed health test is to prevent an infected seed lot to be traded in the market.
- A false negative result may occur due to the poor sensitivity, specificity, or inhibition of a test.
- A false positive resulting from detection of dead or non-infectious viruses could result in an unnecessary rejection of a valuable seed lot.
- Under this situation, particularly with a treated seed lot, the positive result from an indirect test (like ELISA, PCR) should be validated with a direct test to determine virus infectivity, i.e., seedling growout or bioassay, as appropriate.

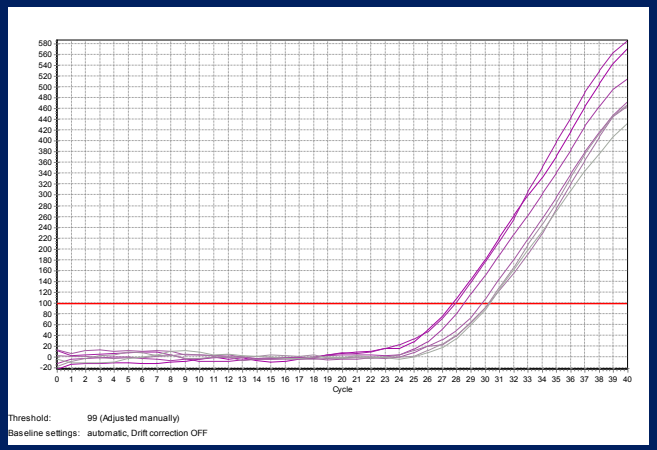
Real-time RT-PCR for specific detection of tobamoviruses, an alternative method or a future for seed health test?

- RNA extraction of seed samples using Trizol reagent.
- Conserved sequences for primer and probe design.
- Using an internal amplification control (i.e., 18S rRNA).
- At least 10 x higher sensitivity than ELISA.

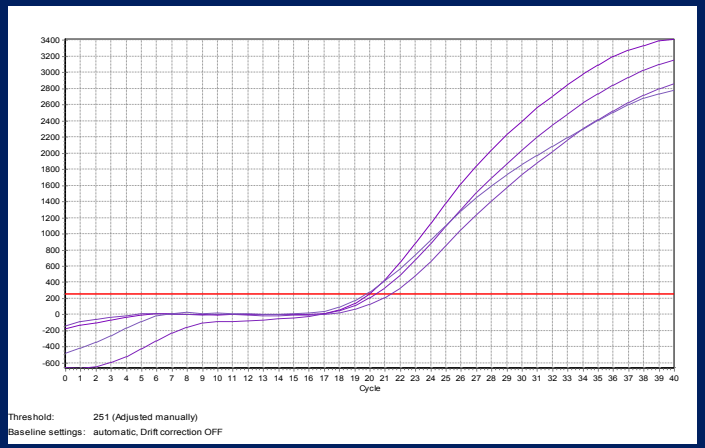
PMMoV



ToMV



TMV



Management of PMMoV

- Host resistance (L^1, L^2, L^3, and L^4 R genes)
- Avoidance (clean seed), seed health test.
- Hygiene, sanitation and disinfection to limit secondary mechanical transmission.
- Rotation to a non-host crop, like tomato, eggplant.

Most Effective Disinfectants for ToMV/TMV

(likely effective for PMMoV too)

10% Bleach

20% NFDM

50% Lysol

Most effective disinfectants against major greenhouse tomato viruses/viroid

(should be useful to manage pepper tobamoviruses)

Disinfectants	PepMV	ToMV	TMV	PSTVd
Clorox (10%)	√	√	√	√
Virkon S (2%)	√	√	√	√
NFD Milk (20%)	√	√	√	√
Lysol (50%)	√	√	√	Partial

Summary

- Planting a certified virus-tested seed lot is an important preventative measure against seed-borne viruses.
- A reliable seed health test should be sensitive in detecting the presence of virus particles, but also need to determine the virus infectivity through appropriate bioassay, particularly on those treated seed lots.
- An integrated disease management should be employed, from planting a virus-resistant cultivar, to the use of effective disinfectants, and crop rotation to non-host plants.

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