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

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The mission of the U.S. Vegetable Laboratory is to improve genetic populations of vegetable crops on yield, quality, and pest resistance; to generate knowledge on the etiology, ecology, epidemiology, and pathogenicity of plant pests, thus to develop new management strategies.
Major vegetable crops that we are working on: Brassica (Broccoli, Cabbage, Cauliflower, and Collard green), Solanaceous crops (Pepper and Tomato), Southern pea, Sweet potato and Watermelon. Today, I will focus my presentation on the emerging tomato diseases.
Coat protein

Detection:

RNA or DNA → Molecular (PCR)

Coat protein → Serological (ELISA)

(Virus structure, composition and the strategies in virus detection)

(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)

**Direct Tests** *(infectious)*
(slow, less sensitive, non-specific)

- **Coat protein**
- **RNA or DNA**

**Tobamovirus** *(Local lesions)*

**SqMV** *(Growout)*
## Major seed-borne viruses and viroids on Solanaceous crops and seed health tests

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Host</th>
<th>Indirect test</th>
<th>Direct test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA</td>
<td>qRT-PCR</td>
</tr>
<tr>
<td><strong>Tobamovirus</strong></td>
<td>PMMoV</td>
<td>Pepper</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TMV</td>
<td>Pepper/tomato</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>ToMV</td>
<td>Pepper/tomato</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>Potexvirus</strong></td>
<td>PepMV</td>
<td>Tomato</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Pospiviroids</strong></td>
<td>PSTVd/P CFVd</td>
<td>Pepper/tomato</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
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)
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.

Negative sample
Positive sample (yellow color)
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.): Interpretaion 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.
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)

<table>
<thead>
<tr>
<th>Disinfectants</th>
<th>PepMV</th>
<th>ToMV</th>
<th>TMV</th>
<th>PSTVd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clorox (10%)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Virkon S (2%)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>NFD Milk (20%)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lysol (50%)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Partial</td>
</tr>
</tbody>
</table>
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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