Guidelines for the Identification of Pepper Tobamovirus Races using Differential Hosts

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Hosts: *Capsicum* spp.
Pathogens: *Tobacco mosaic virus* (TMV)*, Tomato mosaic virus* (ToMV)*, Tobacco mild green mosaic virus* (TMGMV)*, Paprika mild mottle virus* (PaMMV)*, Pepper mild mottle virus* (PMMoV)*, Bell pepper mottle virus* (BPMoV) and *Tomato brown rugose fruit virus* (ToBRFV)

Background:

The Tobamoviruses are readily mechanically transmitted, many are seed transmitted and can also be waterborne (Jacobi and Castello 1991). Within this genus are seven commercially relevant species and strains pathogenic to *Capsicum* spp. As a group, TMV (type species), ToMV, TMGMV, PaMMV, PMMoV, BPMoV and ToBRFV may infect species and cultivars of pepper wherever the crop is grown (Kenyon et. al. 2014, Eldan et. al. 2022). Yield losses can be substantial when resistant cultivars are not available or if resistance is overcome by emerging strains (Moury and Verdin 2012). Mixed infections of PMMoV with TMV and ToMV in pepper are common. Unlike the other pepper Tobamoviruses, TMV and ToMV can also naturally infect tomato, eggplant, and tobacco (Tobias et. al. 1982).

Symptoms in pepper plants include leaf distortion with a mild light green mottle typical of PMMoV (Fig. 1) to a yellow mottle or mosaic typical of ToMV (Fig. 2). Fruit of infected plants may be small, with sunken and raised bumpy areas (Fig. 3) typical of PMMoV to mottled, malformed, or both (Fig. 4) typical of ToMV. Necrotic areas may also be seen in fruit, stems, and leaves. Infected seedlings are often stunted. Symptom severity will vary with infecting virus, pepper genotype, temperature and level of light (Parisi et. al. 2020). Symptoms affect both the quantity and quality of the crop. High titers of the virus are produced in a susceptible pepper host as the virus spreads rapidly through the plant and is subsequently detected in roots, stems, flowers and seeds.

Fig. 1. Mild light green mottle typical of PMMoV

Fig. 2. Yellow mosaic and leaf distortion of ToMV

Fig 3. Bumpy fruit surface typical of PMMoV

Fig. 4. Symptoms on fruit typical of ToMV

The pepper Tobamoviruses have no known insect vector and are readily mechanically transmitted in greenhouse pepper production systems. Infected seeds, fruit, leaves, stems, roots, and plant debris in the soil are common sources of inoculum. Tobamoviruses are stable, rod-shaped particles, 300 x 18 nm in size and consist of a single positive sense RNA molecule surrounded by coat protein subunits in a helical arrangement (Wetter and Conti 1988, Zaitlin and Israel 1965). Because of the coat protein stability, virus particles can survive in dry soil and plant debris for several years (Zitter 1991). Contaminated greenhouse tools and equipment and poor phytosanitation practices also facilitate spread of these viruses (Lewandowski et. al. 2010). The use of seed that has been tested and treated for Tobamoviruses is an important measure to take to help avoid initial infection (Rast and Stijger 1987).

The use of resistant pepper cultivars offers the most effective control of infection spread from a point of origin. Resistance to most Tobamoviruses including ToBRFV (Eldon et. al. 2022) is conferred by four alleles at the L locus on chromosome 11. They are designated L_1, L_2, L_3 and L_4 (Green and Kim 1991, Tenllado et. al. 1994, Rast 1988, Takeuchi et. al. 2005). These resistance alleles initiate a localized necrotic reaction (hypersensitive response) after infection which prevents systemic spread.

Tobamovirus species, groups, and races

The pepper Tobamoviruses are classified into four groups (formerly pathotypes), Tm:0, Tm:1, Tm:2, and Tm:3 based on their responses to the resistance alleles L_1, L_2, L_3 or L_4, respectively. Multiple different Tobamovirus species and races falls into these four groups based on their response to the resistance alleles present in a given pepper host (Table 1). Thus, the code Tm refers to the Tobamovirus group as a whole, not necessarily a specific species or race from within the group.

Serological and PCR methods have also been developed and validated to consistently identify, detect and differentiate the pepper Tobamoviruses.

The International Committee on Taxonomy of Viruses (ICTV) is the standard reference for virus taxonomy. More information on the different Tobamovirus species can be found in the ICTV Master species list of 2023 (https://ictv.global/msl).

Pepper Tobamoviruses and their maintenance: Suspect Tobamovirus isolates must be purified from local lesions. Isolates of known races can be stored in desiccated leaves in a cool, dry environment. Infected plant tissue can also be maintained under liquid nitrogen or at -80C. Cultures can be revived and propagated on a susceptible tomato or tobacco cultivar prior to a test.

Preparation of host plants and inoculum: Pepper seedlings are grown until first true leaves emerge (Fig. 5). Inoculum can be prepared from fresh or frozen infected plant tissue (Fig. 6). Grind in cold phosphate buffer, pH 7.2 - 7.4 (1:10 tissue to buffer weight by volume) until completely homogenized. The buffered inoculum should remain chilled. Abrasive agents (carborundum or celite) may be used to enhance inoculation efficacy.

Fig. 5. Growth stage of seedlings at inoculation

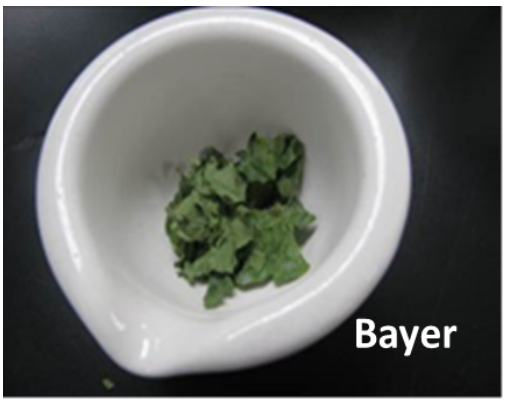

Fig. 6. Symptomatic tissue ready for homogenizing with a mortar and pestle in cold buffer

Inoculation, incubation and evaluation: The cotyledons of tested plants and differential hosts are rub-inoculated (Fig. 7 - 8) with a gloved finger using freshly prepared inoculum as described above. Variations of the test can include inoculation of fully expanded true leaves, which can help with evaluation of the hypersensitive response in resistant cultivars. Inoculated plants are maintained in a greenhouse or growth chamber at 24 to 26°C. Symptoms of the hypersensitive response develop 4 – 7 days after inoculation (Fig. 9) which can lead to cotyledon drop. The hypersensitive response can be difficult to see on fallen cotyledons so monitor inoculated plants daily. Mosaic symptoms of the susceptible response should be visible 3 to 4 weeks after inoculation (Fig. 10). The expected responses across the differential hosts are shown in Table 1.

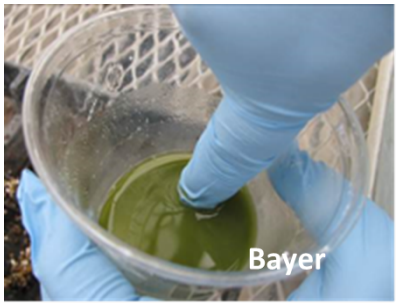

Fig. 7. Dip a gloved finger into inoculum

Fig. 8. Gently rub inoculum onto cotyledon

Fig. 9. Hypersensitive resistant response (arrow)

Fig. 10. Susceptible response

Note: As the virus is very infectious and stable, there is always the risk of cross contamination and so extreme care in performing the test should be observed and all used supplies/materials must be properly decontaminated/disposed (Lewandowski et al., 2010).

Table 1. Expected Responses of the Pepper Tobamoviruses Across Differential Hosts Containing the Four Dominant Single Resistance Genes *L1, L2, L3* and *L4*

		Tobamovirus group			
		Tm:0	**Tm:1**	**Tm:2**	**Tm:3**
Differential Host	R Gene	TMV: 0,1,2 ToMV: 0,1,2 BPMoV	TMGMV PaMMV	PMMoV: 1.2	PMMoV: 1.2.3
Golden Cal Wonder, Early Cal Wonder, Lamu	-	S	S	S	S
Yolo Wonder B, Tisana	*L1*	HR	S	S	S
Tabasco	*L2*	HR	HR	S	S
Novi 3, PI 159236, Solario F1	*L3*	HR	HR	HR	S
PI 260429, CAPMVR, Tom4	*L4*	HR	HR	HR	HR

S = Susceptible; HR = Highly Resistant

*This table is an adaptation of the ISF Differential Hosts table for Pepper Tobamoviruses found here: https://worldseed.org/our-work/disease-resistance/differential-hosts/

**CPPSI provides select seed and isolates representative of each group as listed below.

Differential hosts: Golden Cal Wonder (Susceptible), Yolo Wonder B (*L1*), Tabasco (*L2*), Novi 3 (*L3*), PI 159236 (*L3*), CAPMVR (*L4*), and PI 260429 (*L4*).

Isolates: ToMV: 2 (group Tm:0), PaMMV (group Tm:1), PMMoV: 1.2 (group Tm:2), and PMMoV: 1.2.3 (group Tm:3).

Ordering seeds of differential lines:
Seeds of the differential lines listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network: https://www.ars-grin.gov/). You may search the USDA GRIN database without logging in but cannot order seeds until you create an account and log in to the database.

Type in '**CPPSI***' in the search window. Select the differential hosts to order. Select the cart button at the top of the page to generate an order form. Select 'submit' to place your order.

A limited supply of **50-100 seeds per differential** can be ordered at no charge, if adequate seed is in supply. The USDA National Plant Germplasm System in which the GRIN database is housed may not always have adequate seed of all the differentials listed to provide a full set of differentials.

If you have difficulties ordering seeds, contact Kelley Clark at kjclark@ucdavis.edu for assistance.

Ordering races of the pathogen
Reference races of this plant pathogen can be ordered from Amy Gurza or Andy Hagan. **Please have valid USDA-APHIS permit or other import permits ready in order to receive reference isolates.**

amy.gurza@usda.gov
andy.hagan@usda.gov

Amy phone: 970-492-7554
Andy phone: 970-492-7555

National Lab for Genetic Resources Preservation Unit
1111 South Mason St.
Fort Collins, CO 80521

Feedback
Inquiries on how to participate and support CPPSI, provide feedback on new races identified, views on the inoculation protocols, differential hosts, or any related matter is welcomed. Please contact: Kelley Clark at kjclark@ucdavis.edu.

Liability waiver
The CPPSI Collaboration for Plant Pathogen Strain Identification, USDA NPGS/GRIN, APS, ASTA, and all other associated members and participating organizations or companies have done their best to provide information that is up-to-date and published in refereed journals and, therefore, no liability for the use of this information is accepted. The inoculation protocol described in this document has been demonstrated to be effective at identifying strains of Tobamoviruses and resistance traits of listed pepper cultivars.

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