

Collaboration for Plant Pathogen Strain Identification

GUIDELINES FOR IDENTIFICATION OF *TOMATO MOSAIC VIRUS* STRAINS USING DIFFERENTIAL HOSTS

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Host: *Solanum lycopersicum*

Pathogen: *Tomato mosaic virus* (ToMV)

Background

Tomato mosaic virus (ToMV) and *Tobacco mosaic virus* (TMV) are members of the *Tobamoviruses*. These two viruses are highly infectious, attain high concentrations in all infected tissues, can survive in plant debris for varying periods and are readily spread through cultivation practices. Both viruses have a wide host range including many agricultural crops and weeds, all of which can serve as inoculum sources. Insects with chewing mouthparts have been shown to transmit TMV and ToMV but are not considered a significant source of infection or disease spread. Much of the work reported on TMV in the early literature was done before ToMV was described as a separate *Tobamovirus* species.

TMV and ToMV can be differentiated serologically or based on nucleic acid sequence, but not by phenotypic reactions across differential tomato hosts containing the Tm resistance genes. However, it is reported that TMV infects white burley tobacco, *Nicotiana sylvestris* and *Datura stramonium* systemically while ToMV causes local lesions in these hosts (Hollings and Huttinga 1976). When using these differential host sets to identify unknown tomato *Tobamovirus* strains, either virus can be used as a reference isolate.

TMV and ToMV are seedborne and seed transmitted (Broadbent, 1965; 1976; Chitra et al 1999; Gooding and Suggs, 1976). ToMV has been a problem in seed and fruit production of tomato, *Solanum lycopersicum* (formerly *Lycopersicon esculentum*), and pepper, *Capsicum annuum*. The disease can be especially severe during transplant production in greenhouses when resistant cultivars are not used and established phytosanitation practices are not properly followed (Broadbent, 1965 and 1976; Gooding and Suggs, 1976).

Symptoms of ToMV expressed in tomato plants are highly variable and can depend on which strain infects this host, age of the host at infection and the prevailing environmental conditions. Foliar symptoms include a light green to a bright yellow mottling or mosaic (Fig. 1 – 2), upward leaf rolling and/or a severe attenuation or 'fern-leaf' appearance (Fig. 3). Dark longitudinal streaks of varying lengths can be found on stems. Fruit may show uneven coloring and often ripen unevenly (Fig. 4) with internal browning of the fruit walls ('brown wall'). High temperatures can mask foliar symptoms (Pelham, 1966; Pilowsky et al., 1981).



Fig. 1. Light green – dark green mosaic



Fig. 2. Yellow mosaic



Fig. 3. Attenuated and shoestring leaves in susceptible plants



Fig. 4. Uneven coloring in fruit

Strains of *Tomato mosaic virus*

In tomato, naming of the four strains of ToMV currently recognized (ToMV: 0, ToMV: 1, ToMV: 2 and ToMV: 2²) is based on the introgressed resistance (R) genes *Tm1*, *Tm2* and *Tm2*² from related wild species (Pelham, 1966; Hall, 1980). The *Tm1* gene was introgressed from *Solanum habrochaites* (formerly *Lycopersicon hirsutum*) and is incompletely dominant. The *Tm2* and *Tm2*² genes introgressed from *Solanum peruvianum* (formerly *Lycopersicon peruvianum*), are considered allelic and confer dominant complete resistance (Lanfermeijer et al., 2003; Pelham, 1966; Schroeder et al., 1967; Hall, 1980).

New strains of ToMV have emerged as resistance is overcome (Gebre-Selassie and Marchoux, 2008; Komuro et al., 1966; Stoimenova, 1995). Until now, resistance-breaking ToMV strains had not been reported in nature (Garcia-Arenal and McDonald, 2003; Hall, 1980; Pelham et al., 1970). In 2013, a new Tobamovirus was found

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in commercial fields in Mexico (Li et al. 2013), Florida (Webster et al. 2014) and China (Li et al. 2014). The virus was characterized as *Tomato mottle mosaic virus* (ToMMV) and is closely related to ToMV. Anecdotal information questions whether or not ToMMV is consistently controlled by the *Tm2²* gene in varying backgrounds (Ling 2015), however additional non published studies demonstrated control of ToMMV in many cultivars with *Tm2²* resistance. The virus has also been found in Brazil, Iran and Israel (Turina et al. 2015). While the host range of ToMMV is limited to tomato and pepper (*Capsicum annuum*), the virus has been found in California and the California Department of Food and Agriculture rates the risk of spread as high because this virus is stable and can spread in a manner similar to ToMV where tomatoes and peppers are grown.

Identification of *Tomato Mosaic Virus* Strains Using Host Differentials

ToMV strains and their maintenance - Suspect ToMV isolates or strains must be purified from local lesions. Strains are stored in desiccated leaves in a cool, dry environment. A strain can be revived and propagated on a susceptible tomato or tobacco cultivar prior to a test.

Preparation of host plants and inoculum - Tomato seedlings are grown until the first-true leaf is emerging (Fig. 5). Inoculum is prepared by flash freezing fresh symptomatic plant tissue with liquid nitrogen (Fig. 6), then homogenizing in cold phosphate buffer, pH 7.2 - 7.4 (1:10 tissue to buffer weight by volume). The buffered inoculum should remain chilled. Abrasive agents (carborundum or celite) may be used to enhance inoculation efficacy.



Fig. 5. Tomato seedlings, first true leaf emerging



Fig. 6. Frozen fresh symptomatic tissue to be homogenized with a mortar and pestle in cold buffer

Inoculation and Incubation - The cotyledons of test plants are rub-inoculated (Fig. 7 - 8) with a gloved finger using freshly prepared inoculum as described above. Rinse the plants after inoculation. Maintain inoculated plants in a greenhouse or growth chamber at 24 to 26°C. Symptoms are enhanced by low light levels. Symptoms should be evaluated 10 to 14 days after inoculation (Pilowsky et al., 1981; Schroeder et al., 1967).



Fig. 7. Dip a gloved finger into inoculum mixture



Fig. 8. Gently rub inoculum mixture onto cotyledons

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Table 1. Expected reactions of tomato differential hosts to strains of *Tomato mosaic virus* (ToMV)

| Strain | Host Differential | | | |
|----------------------|-------------------|-----------------------|----------------------------|---|
| | Early Pak 7 | Mobaci (<i>Tm1</i>) | Moperou 161 (<i>Tm2</i>) | Momor, Geneva 80, (<i>Tm2</i> ²) |
| ToMV: 0 | S | R/IR | HR | HR |
| ToMV: 1 | S | S | HR | HR |
| ToMV: 2 | S | R/IR | S | HR |
| ToMV: 1.2 | S | S | S | HR |
| ToMV: 2 ² | S | R/IR | HR | S |

S = Susceptible IR = Intermediately Resistant HR = Highly Resistant

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).

Evaluation of inoculated plants - The test plants are evaluated 10 to 14 days after inoculation based on the appearance of symptoms on the foliage above the inoculated leaves. Symptoms can be evaluated following established criteria (Pelham, 1966; Pelham et Al. 1970; Pilowsky et al., 1981). Generally, susceptible plants are stunted and develop mosaic symptoms on leaves (Fig. 9 - 11). Plants with *Tm2* and *Tm2*² are generally symptomless (Fig. 9, 10, 12).



Fig. 9. Stunting in susceptible seedlings (L) Resistant plants (R)

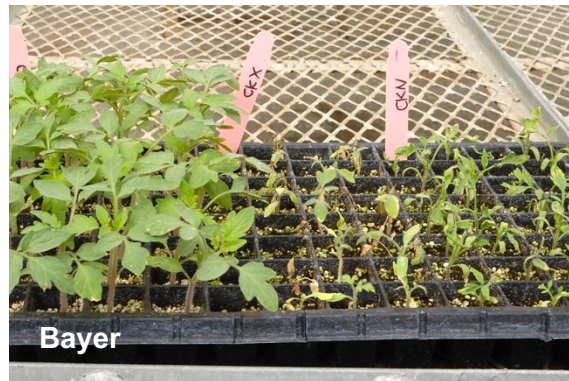


Fig. 10. Resistant (L); Heterozygous (M); and Susceptible (R) reactions



Fig. 11. Close up of susceptible response



Fig. 12. *Tm2* and *Tm2*² resistant plants

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Fig. 13. Heterozygous reaction



Fig. 14. Mild mosaic and localized necrotic lesions in hybrid cultivars heterozygous for *Tm* genes

It is important to understand that *Tm1* confers incomplete or partial resistance. Symptoms will be obvious when compared to non-inoculated plants, but less severe than those seen on inoculated, susceptible plants.

A systemic hypersensitive reaction (i.e. the virus moves systemically) may occur in seedlings. While the seedlings die, the evaluated line or hybrid is resistant. Under stressed conditions (high or low temperatures and / or light), a mild mosaic and localized necrotic lesions may also occur in hybrid cultivars that are heterozygous for the *Tm* genes (Fig. 10, 13, 14).

Under stressed conditions, symptoms of brown to grey discoloration may appear on and in fruits of hybrid ToMV resistant cultivars (Fig. 15).



Fig. 15. Brown to grey discoloration on and in fruits of stressed hybrid ToMV resistant cultivars

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Ordering seeds of differential lines

Seeds of each 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 seeds per differential can be ordered at no charge, as long as there is adequate seed 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 strains of the pathogen

Reference strains of ToMV: 0, ToMV: 1, and ToMV: 1.2 can be ordered from the National Center for Genetic Resources Preservation via the online GRIN system. Follow the same GRIN access instructions for ordering seeds.

For questions regarding these reference races, contact:

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 ToMV and TMV and resistance traits of tomato cultivars.

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Related websites

<http://www.cpvo.europa.eu/main/en/home/technical-examinations/technical-protocols/tp-vegetable-species>

http://www.worldseed.org/isf/pathogen_coding

<http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB>

UC Davis Tomato Genetics Resource Center <http://tgrc.ucdavis.edu/>

National Plant Disease Network <https://www.npdn.org>

Plant Management Network <https://www.plantmanagementnetwork.org>