

Isolation and Partial Characterization of a Tobamovirus from Flowering Dogwood in Tennessee

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ABSTRACT

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One hundred forty-five flowering dogwood trees (*Cornus florida*) used as propagative material were assayed by mechanical inoculation to herbaceous test plants for virus incidence. Only one virus was isolated from one tree, which upon examination with an electron microscope was found to have tobamoviruslike particles. This virus, designated ToMV-DW, was compared with known tobacco mosaic, tobacco mild green mosaic, and tomato mosaic virus isolates using particle morphology, host-range studies, serological tests, and whole virion gel electrophoresis. ToMV-DW was found to be a distinct isolate of tomato mosaic virus.

There have been six viruses (dogwood mosaic, broad bean wilt, cherry leaf roll, cucumber mosaic, tobacco ringspot, and tomato ringspot) previously reported from flowering dogwood (*Cornus florida* L.) (3). In Tennessee, the sale of flowering dogwood is an important part of the more than \$200 million per year nursery sales with about \$30-35 million annually (Ken Tilt, *personal communication*). In many instances, the dogwood stock trees are used for vegetative propagation. In 1984, a survey of 145 trees in eight nurseries was conducted to assess virus incidence by sap-inoculation to herbaceous test plants from some of these dogwood stock blocks in Tennessee. Virus isolates were obtained from three of the 145 dogwood trees sampled. Two of these virus isolates could not be reisolated from dogwood. The third isolate was reisolated from a symptomless dogwood and, upon examination with an electron microscope, was shown to have particles resembling those of the tobamovirus group.

This paper reports the characterization of this tobamovirus isolated from flowering dogwood referred to as ToMV-DW.

MATERIALS AND METHODS

Virus isolates. Tobacco mild green mosaic virus isolate U₂ (TMGMV-U₂) and tomato mosaic virus (ToMV) isolates L (ToMV-L) and Dahlemense (ToMV-Dahlemense) and antiserum against the TMGMV-U₂ isolate were obtained from M. Zaitlin (Cornell University). Tobacco mosaic virus U or type isolate (TMV-U₁) and antiserum were obtained from J. S. Sherwood (Oklahoma State University). TMGMV-

228 isolate was from the American Type Culture Collection (Rockville, MD).

Inoculations and host range. Leaves or bracts from dogwood trees were ground in 2% nicotine (1:2, w/v) and the sap was used to inoculate *Chenopodium quinoa* Willd. and *Nicotiana clevelandii* A. Gray using the Carborundum gauze-pad method. Subsequent inoculations to plants used for virus maintenance and host-range studies were made as above except, instead of 2% nicotine, 0.03 M sodium phosphate buffer (pH 7.2) was used. Back inoculations from symptomless host-range plants to *N. glutinosa* L. were performed 14-18 days postinoculation. All TMV, TMGMV, and ToMV isolates were carried through single-lesion inoculation series five to seven times using *N. glutinosa* as the local lesion host. ToMV-DW was inoculated to dogwood seedlings, as above, in 0.03 M sodium phosphate buffer.

Purification. All TMV, TMGMV, and ToMV virions were partially purified from *N. tabacum* L. 'Judy's Pride', according to the methods of Gooding and Hebert (4). The virions were further purified by centrifugation on a 10-40% sucrose density gradient in 0.01 M sodium phosphate buffer (pH 7.0) for 2 hr at 97,000 g. Each virus band was collected and concentrated by high-speed centrifugation for 2 hr at 134,000 g and resuspended in a small volume of 0.01 M sodium phosphate buffer (pH 7.0).

Electron microscopy. Purified virion preparations of ToMV-DW and TMV-U₁ were spread on Formvar-carbon coated 300-mesh copper grids, stained with 2% phosphotungstic acid (pH 7.0), and examined using a Philips 300 electron microscope.

Serology. Antisera to isolates ToMV-DW, ToMV-L, ToMV-Dahlemense, and TMGMV-228 were produced by rabbits injected, both subcutaneously and

intramuscularly at 3 weekly intervals, with 1 mg of purified virions in 1 ml of 0.01 M phosphate buffer (pH 7.0) plus 1 ml of Freund's incomplete adjuvant. A booster injection was given 2 wk following the last of the three injections. Rabbits were bled at weekly intervals starting 1 wk after the booster injection.

Double-diffusion serology tests (7) were conducted with 0.6% Ionagar No. 2 (Colab Laboratories, Inc., Glenwood, IL) in phosphate-buffered saline (0.02 M phosphate, 0.15 M NaCl, 0.02% sodium azide [pH 7.3]) using purified virions. Intragel cross-absorption tests were performed according to Van Regenmortel (7) using 0.6% Ionagar No. 2, 0.85% sodium chloride, and 0.25% sodium azide. The absorbing virions (2 mg/ml) were placed in the center well 24 hr before TMV, TMGMV, or ToMV antisera (1:5 dilution with saline) and the individual TMV, TMGMV, and ToMV isolates (2 mg/ml) were placed in the outer wells of the double-diffusion plates.

Gel electrophoresis. Whole TMV, TMGMV, and ToMV virions were subjected to electrophoresis as described by Asselin and Grenier (1). This technique has been used to separate tobamovirus isolates into subgroups and to evaluate and enhance the purity of tobamovirus isolates (1,2). Purified virions (50 µg in 20 µl) were electrophoresed (25 V, 50 mA overnight) through 1.2% (w/v) agarose in 40 mM sodium borate buffer (pH 8.0) containing 0.25 mM EDTA and 0.25 M urea. Virion bands were stained with 0.1% Coomassie Brilliant Blue R-250 for 1 hr and destained with 20% methanol and 6% acetic acid.

RESULTS

Host range and symptomatology. The reactions of the host-range plants to inoculation of five tobamovirus isolates (TMV-U₁, TMGMV-U₂, ToMV-DW, ToMV-L, and ToMV-Dahlemense) are listed in Table 1. The TMV-U₁ and TMGMV-U₂ isolates could be differentiated from the two reference tomato mosaic isolates and ToMV-DW in that the TMV-U₁ and TMGMV-U₂ isolates caused local lesions in *Phaseolus vulgaris* L. 'Black Turtle I', 'Black Turtle II', and 'Pinto', whereas the ToMV-L, ToMV-DW, and ToMV-Dahlemense did not. The isolates TMV-U₁ and TMGMV-U₂ also caused systemic mosaic symptoms in

Table 1. The response of selected host plants to five tobamovirus isolates

Host species	Tobamovirus isolate ^a				
	TMV-U ₁	TMGMV-U ₂	ToMV-L	ToMV-Dahlemense	ToMV-DW
<i>Capsicum annuum</i>					
cv. California Wonder	CL/M ^b	-/N	CL,NL,Epi/M	CL,NL/Epi,M	NL/-
cv. Jalapeno	CL/M	-/M	CL/Epi,M,N	NL/M	CL,NL/N,Stu
cv. Long Red Cayenne	NL/M	-/M	CL/M,N,Stu	CL,NL,Epi/M,N	NL/M,N,Stu
cv. Pimento	-/M	-/M	CL/M,N	CL,NL,Epi/M,N	NL/M,N
<i>Lycopersicon esculentum</i>					
cv. Floradade	-/-	-/M	-/M	-/M	CL/-
<i>Phaseolus vulgaris</i>					
cv. Black Turtle I	RL/-	RL/-	-/-	-/-	-/-
cv. Black Turtle II	RL/-	RL/-	-/-	-/-	-/-
cv. Pinto	RL/-	RL/-	-/-	-/-	-/-
<i>Nicotiana glutinosa</i>	CL/-	NL/-	NL/-	NL/-	NL/-
<i>N. rustica</i>	CL/M	CL/M	NL/-	NL/-	NL/-
<i>N. sylvestris</i>	-/M	NL/-	NL/-	NL/-	NL/-
<i>N. tabacum</i>					
cv. Judy's Pride	-/M	CL/M	-/M	-/M	CL/M
cv. Tennessee 86	CL/M	CL/M	NL/-	NL/-	NL/-
cv. Virginia 509	-/M	NL/M	NL/-	NL/-	NL/-
cv. White Burley	NL/M	NL/M	NL/-	NL/-	NL/-

^aStandard isolates of tobacco mosaic virus (TMV), tobacco mild green mosaic virus (TMGMV), and tomato mosaic virus (ToMV). ToMV-DW was isolated from dogwood from Tennessee.

^bLocal symptoms/systemic symptoms. CL = chlorotic lesion, Epi = epinasty, M = mosaic, N = necrosis, NL = necrotic lesion, RL = red lesion, Stu = stunting, and - = no local or systemic symptoms and no systemic infection, as determined by inoculation to a local lesion host.

N. rustica L. and in *N. tabacum* 'Virginia 509', 'Tennessee 86', and 'White Burley', whereas with the reference ToMV isolates and ToMV-DW no systemic infection occurred. *N. sylvestris* Speg. & Comes was systemically infected only by TMV-U₁. ToMV-DW could be differentiated from ToMV-L and ToMV-Dahlemense in that it did not systemically infect *Capsicum annuum* L. 'California Wonder' or *Lycopersicon esculentum* Mill. 'Floradade', whereas ToMV-L and ToMV-Dahlemense did. ToMV-DW caused local chlorotic lesions in *N. tabacum* 'Judy's Pride', but ToMV-L and ToMV-Dahlemense did not. ToMV-L, ToMV-DW, and ToMV-Dahlemense caused systemic mosaic symptoms in 'Judy's Pride'. ToMV-Dahlemense could be differentiated from ToMV-DW and ToMV-L in that local symptoms were observed in *Vicia faba* L. only when inoculated with ToMV-Dahlemense. The reaction of *C. annuum* 'Pimento', 'Jalapeno', and 'Long Red Cayenne' to ToMV-DW, ToMV-L, and ToMV-Dahlemense differed somewhat for each isolate. However, symptomatology was variable between individual plants, and therefore these pepper cultivars did not consistently differentiate the three isolates.

ToMV-DW was recovered from one of two inoculated, but symptomless, dogwood seedlings when tested by back inoculation to *N. glutinosa*.

Electron microscopy. Average particle length for ToMV-DW and TMV-U₁ was 296 ± 18.5 nm and 306 ± 18.5 nm, respectively (200 virions measured). Preparations of both isolates had some broken or short particles, and several particles showed end-to-end aggregation.

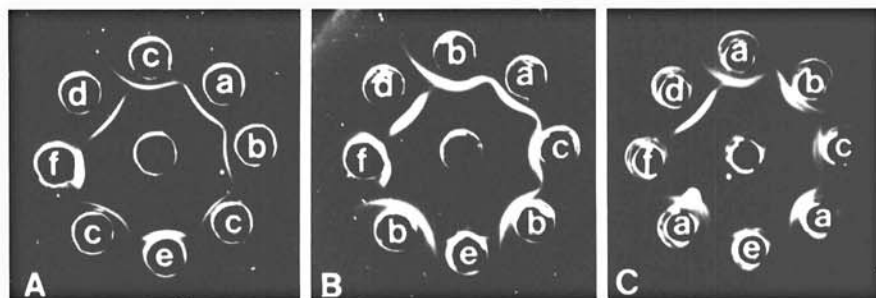


Fig. 1. Results of immunodiffusion tests using purified virions (2 mg/ml) and antisera to various tobamovirus isolates. Peripheral wells contained virions of: a = ToMV-Dahlemense, b = ToMV-L, c = ToMV-DW, d = TMGMV-228, e = TMV-U₁, and f = TMGMV-U₂. Central wells contained (A) ToMV-DW antiserum, (B) ToMV-L antiserum, and (C) ToMV-Dahlemense antiserum.

Little difference was seen between preparations or isolates.

Serology. In agar gel double-diffusion tests, ToMV-DW was serologically related to TMV-U₁, TMGMV-U₂, TMGMV-228, ToMV-L, and ToMV-Dahlemense. Spur formation was observed between ToMV-DW and TMV-U₁, TMGMV-U₂, and TMGMV-228 when the antiserum to ToMV-DW was used. However, no spur formation occurred between ToMV-DW and ToMV-L or ToMV-Dahlemense (Fig. 1A). When antiserum to ToMV-L (Fig. 1B) was used, spur formation was observed between ToMV-L and TMV-U₁, TMGMV-U₂, and TMGMV-228. No spurs were observed between ToMV-L and ToMV-Dahlemense or ToMV-DW. However, a second distinct precipitin line was observed. Antiserum to ToMV-Dahlemense yielded a more complex precipitin pattern when reacted to the tobamovirus isolates mentioned above (Fig. 1C). Spur formation was observed between ToMV-Dahlemense and TMV-U₁ and TMGMV-U₂. One continuous

precipitin line occurred between the homologous virus isolate and TMGMV-228, behind which a second curved precipitin line formed with a long curved spur. This suggests that ToMV-Dahlemense shares at least one antigenic site with TMGMV-228 that was not observed with antiserum to either ToMV-DW or ToMV-L. Antiserum to ToMV-Dahlemense could also be used to distinguish between ToMV-DW and ToMV-L, in that spur formation was observed between these two isolates. Similar patterns were observed with two different antisera to ToMV-Dahlemense.

Results of the intragel cross-absorption tests are listed in Table 2. These results agree with the double-diffusion tests, in that antisera against either ToMV-DW or ToMV-L were unable to distinguish serologically between ToMV-DW, ToMV-L, or ToMV-Dahlemense. However, anti-Dahlemense serum did react differently when first absorbed with ToMV-L or ToMV-DW. When ToMV-L was used as the absorbing virus followed by anti-ToMV-Dahlemense serum, no precipitin lines were formed, but if ToMV-DW was

Table 2. Intragel cross-absorption test of six tobamovirus isolates

Antiserum	Antigen used for absorption ^a	Tobamovirus antigen ^b					
		ToMV-DW	ToMV-L	ToMV-Dahlemense	TMV-U ₁	TMGMV-U ₂	TMGMV-228
ToMV-DW	ToMV-L	— ^c	—	—	—	—	—
ToMV-DW	ToMV-Dahlemense	—	—	—	—	—	—
ToMV-DW	TMGMV-U ₂	+	+	+	—	—	+
ToMV-DW	TMV-U ₁	+	+	+	—	—	+
ToMV-DW	TMGMV-228	+	+	+	+	+	—
ToMV-L	ToMV-DW	—	—	—	—	—	—
ToMV-L	ToMV-Dahlemense	—	—	—	—	—	—
ToMV-L	TMGMV-228	+	+	+	+	+	—
ToMV-Dahlemense	ToMV-DW	—	+	+	—	—	—
ToMV-Dahlemense	ToMV-L	—	—	—	—	—	—
ToMV-Dahlemense	TMGMV-228	+	+	+	+	+	—

^aThe absorbing antigen (2 mg/ml) was placed in the center well 24 hr before the addition of the antisera (1:5 dilution with saline) and the antigens (2 mg/ml) in the surrounding wells.

^bStandard isolates of tobacco mosaic virus (TMV), tobacco mild green mosaic virus (TMGMV), and tomato mosaic virus (ToMV). ToMV-DW was isolated from dogwood from Tennessee.

^c— = No precipitin lines formed between the antiserum and the challenging antigen, and + = a precipitin line formed between the antiserum and the challenging antigen.

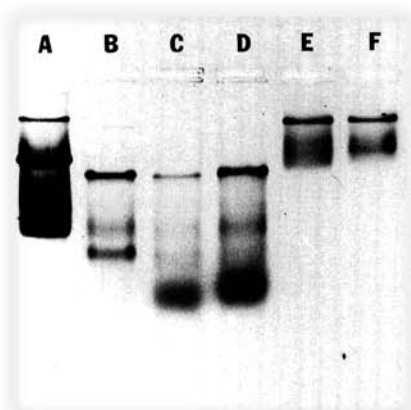


Fig. 2. Electrophoretic mobility in 1.2% agarose gel of virions of five standard tobamovirus isolates described in the text and the isolate from a dogwood tree (ToMV-DW) in Tennessee (lane A = TMV-U₁, lane B = ToMV-DW, lane C = ToMV-L, lane D = ToMV-Dahlemense, lane E = TMGMV-228, and lane F = TMGMV-U₂). Virions were subjected to electrophoresis at 25 V and 50 mA overnight in 40 mM sodium borate buffer (pH 8.0) containing 0.25 mM EDTA and 0.25 M urea. Staining was with Coomassie Brilliant Blue R-250.

the absorbing virus, precipitin lines formed between ToMV-L, ToMV-Dahlemense, and the ToMV-Dahlemense antisera.

One of the two dogwood seedlings inoculated with ToMV-DW gave a positive serological reaction when reacted against ToMV-DW antiserum.

Gel electrophoresis. Electrophoresis patterns of the tobamovirus virions can be seen in Figure 2. Each isolate has an individual profile. However, TMV-U₁, TMGMV-U₂, and TMGMV-228 were distinctly different from the tomato mosaic virus isolates (ToMV-DW, ToMV-L, and ToMV-Dahlemense). ToMV-L and ToMV-Dahlemense had almost identical profiles, with the largest

band for each migrating much further from the origin than either ToMV-DW, TMV-U₁, TMGMV-U₂, and TMGMV-228. ToMV-DW shares two bands with ToMV-L and ToMV-Dahlemense, but differs in the position of the largest band.

DISCUSSION

ToMV-DW, isolated from *C. florida*, is a distinct isolate of tomato mosaic virus (5,8,9) because of biological, serological, and physical differences observed when compared with ToMV-L and ToMV-Dahlemense. ToMV-DW was successfully inoculated to, and recovered from, a dogwood seedling, and was isolated twice from its original naturally infected dogwood tree, indicating that ToMV-DW was not a greenhouse contaminant. The particle size indicates that ToMV-DW is a tobamovirus. The host range and symptomatology of ToMV-DW were similar to ToMV-L and ToMV-Dahlemense. However, a few differences were observed. The host range of ToMV-DW differed greatly from the tobamoviruses TMV-U₁ and TMGMV-U₂. Serologically, ToMV-DW could not be distinguished from ToMV-L or ToMV-Dahlemense when reacted against ToMV-DW or ToMV-L antiserum in either double-diffusion or intragel cross-absorption tests. Precipitin patterns using antisera to ToMV-Dahlemense were more complex. Double-diffusion precipitin patterns indicate that ToMV-Dahlemense antisera reacts differently with ToMV-DW and ToMV-L in that a spur was formed between these isolates (Fig. 1C). In intragel cross-absorption tests, ToMV-DW and ToMV-L also reacted differently to anti-ToMV-Dahlemense serum (Table 2). Van Regenmortel noted a difference in serological relationships among tobamovirus isolates using intragel cross-absorption, depending on which way the

relationship was tested, suggesting differences in immunogenicity and reactivity of epitopes (6). Differences in rabbits used to produce polyclonal sera must also be taken into account. For the purpose of this paper it is sufficient to note that ToMV-DW is serologically more closely related to ToMV-L and ToMV-Dahlemense than to TMV-U₁, TMGMV-U₂, or TMGMV-228. ToMV-DW was distinct in its electrophoresis pattern of whole virus from any of the isolates tested, but was closest in the pattern to ToMV-L and ToMV-Dahlemense. This is the first report of naturally occurring tomato mosaic virus from flowering dogwood.

LITERATURE CITED

- Asselin, A., and Grenier, J. 1985. Detection of tobamoviruses from purified or crude extracts after agarose gel electrophoresis. *Can. J. Plant Pathol.* 7:223-227.
- Asselin, A., Sevigny, P., and Houge, R. 1984. A note on the preparation of electrophoretically purified isolates of tobacco mosaic virus. *Phytoprotection* 65:84-88.
- Barnett, O. W., Reddick, B. B., and Baxter, L. W. 1989. Characterization of dogwood mosaic nepovirus from *Cornus florida*. *Phytopathology*. In press.
- Gooding, G. V., Jr., and Hebert, T. T. 1967. A simple technique for purification of tobacco virus in large quantities. *Phytopathology* 57:1285.
- Hollings, M., and Huttinga, H. 1976. Tomato mosaic virus. No. 156 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
- Reddick, B. B., Barnett, O. W., and Baxter, L. W., Jr. 1979. Isolation of cherry leafroll, tobacco ringspot and tomato ringspot viruses from dogwood trees in South Carolina. *Plant Dis. Rep.* 63:529-532.
- Van Regenmortel, M. H. V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. *Virology* 31:467-480.
- Wetter, C. 1986. Tobacco mild green mosaic virus. Pages 205-219 in: *The Plant Viruses*. Vol. 2. M. H. V. Van Regenmortel and H. Fraenkel-Conrat, eds. Plenum Press, New York.
- Zaitlin, M., and Israel, H. W. 1975. Tobacco mosaic virus. No. 151 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.