A Mild Strain of Tomato Aspermy Virus Isolated from Tomato in Maryland

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ABSTRACT

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A virus 30 nm in diameter and serologically related to the tomato aspermy virus (TAV) type strain was isolated from volunteer tomato plants in Prince George's County, MD. The isolate has been designated TAV-M. *Spinacia oleracea* 'Bounty' (spinach) was a suitable propagative host, yielding 1–5 mg of virus per 100 g of leaf tissue 12–14 days after inoculation. *Chenopodium quinoa* was a reliable assay host, producing necrotic local lesions 6–9 days after inoculation. The longevity in vitro of TAV-M was between 30 and 48 hr at 25 C, the thermal inactivation point was between 60 and 65 C, and the dilution end point was between 10^{-4} and 10^{-5} in Bounty spinach. TAV-M did not produce enations typical of TAV or fruit aspermy in tomato plants but temporarily inhibited growth and caused a mild leaf mottle of inoculated seedlings. *Arachis hypogaea* (peanut) and *Zea mays* (corn) were infected by TAV-M without showing symptoms.

Tomato aspermy virus (TAV) belongs to the cucumovirus group that also includes cucumber mosaic virus (CMV) and peanut stunt virus (PSV). The virus was originally described by Blencowe and Caldwell in 1949 (2). Although the virus was isolated from tomato (*Lycopersicon esculentum* Mill.), the authors stated that the virus may have been transmitted to tomatoes from chrysanthemum stocks (*Chrysanthemum morifolium* Ramat.: Hemsl.). Aspermy viruses have continued to be called TAV despite the fact that most isolates are recovered from chrysanthemum, in which they cause severe flower break or distortion (8). Type TAV causes severe systemic leaf mottle, enations, dwarfing, and seedlessness or aspermy in infected tomato plants (2).

Serological relationships have been demonstrated among strains of the cucumovirus group (19), but the degree of serological relatedness among members of the group has been interpreted differently by various authors (3,5,6, 10,12,18).

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In 1980, we found a group of volunteer tomato plants that displayed viruslike symptoms in a garden border in Prince George's County, MD. The plants were assayed onto a number of indicator hosts in the greenhouse and were found to contain a virus with a host range typical of the cucumoviruses. This study was initiated to identify the causal agent. A preliminary report has been made of isolation of the virus (9).

MATERIALS AND METHODS

The TAV-M culture was maintained and multiplied in tomato plants (cultivar Walter). *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. were used as assay hosts. Mechanical transmissions were made by grinding infected leaves in 0.1 M sodium-potassium phosphate buffer (pH 7.6) and inoculating test plants dusted with 600-mesh Carborundum. Symptomless test plants were assayed back to the indicator hosts.

Physical properties of the virus were determined using leaves of *Spinacia oleracea* L. (1:1, w/v) triturated in 0.1 M phosphate buffer, pH 7.6. Longevity in vitro, thermal inactivation point, and dilution end point were determined according to methods of Noordam (14).

TAV-M was purified from *S. oleracea* following the method of Mossop et al (13). Purified preparations of the virus were placed on 400-mesh Formvar-coated grids and negatively stained with phosphotungstate (pH 5.0) for observation with the electron microscope. Particle measurements were made on more than 600 particles from different purifications at a nominal magnification of 4×10^4.

Antiserum was produced against TAV-M by intravenous injections of 1 mg of purified virus into New Zealand white rabbits. Two intramuscular injections of 1 mg of purified virus in 0.5 ml of Freund's incomplete adjuvant were given at 2-wk intervals. Blood was collected from the marginal ear veins. Reactivity between TAV-M antigen and homologous antiserum and heterologous antisera TAV 161, PSV 136 (Virginia isolate), CMV 260 (CMV-D), and CMV 242a (CMV-S) (American Type Culture Collection, Rockville, MD) was determined by microprecipitation and tube precipitation tests. Immunodiffusion tests were made in 0.7% Ionagar no. 2 in 0.1 M phosphate buffer, pH 7.6, and 0.02% NaN_3. The type TAV antiserum was used in tests for serological relatedness.

RESULTS

C. quinoa and *C. amaranticolor* were useful as assay hosts. *C. quinoa* produced abundant necrotic local lesions 6–9 days after inoculation and was a more reliable assay host than *C. amaranticolor*. TAV-M produced various types of systemic symptoms (leaf mottling, distortions, and mosaic) on inoculated leaves of *Helianthus annuus* L., *Chrysanthemum morifolium*, *Zinnia elegans* Jacq., *Datura stramonium* L., *Phaseolus vulgaris* L., *Phlox drummondii* Hook., *Nicotiana tabacum* ('Samsum nn' and 'Turkish') L., *N. glutinosa* L., *N. clevelandii* A. Gray, *Petunia hybrida* Vilm., *Physalis floridana* Rydb., *Salpiglossis sinuata* Ruiz & Pav., and *Spinacia oleracea* (Fig. 1).

Chlorotic lesions developed and TAV-M was recovered from inoculated leaves of *Amaranthus caudatus* L., *Gomphrena globosa* L., and *Emilia sagittata* Vahl.: DC. Necrotic local lesions developed on *C. amaranticolor*, *C. quinoa*, *Beta*

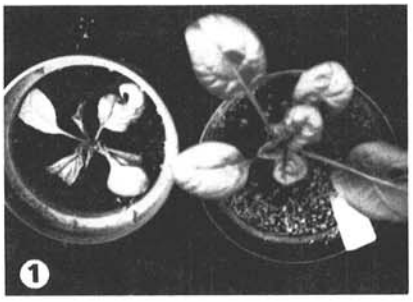

Fig. 1. Comparison of (right) healthy spinach seedling and (left) seedling infected with mild TAV strain at the six-leaf stage, 14 days after inoculation.

vulgaris L., *Vigna unguiculata* L.: Walp., and *Apium graveolens* L. Chlorotic lesions were observed and virus was recovered from the cotyledons but not from the inoculated leaves of *Cucumis sativus* L. Species on which symptoms were not produced but from which virus was recovered included *Arachis hypogaea* L. and *Zea mays* L.

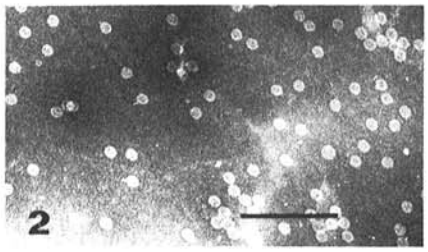

Fig. 2. Electron micrograph of virus particles negatively stained with 2% potassium phosphotungstate. Scale bar = 200 nm.

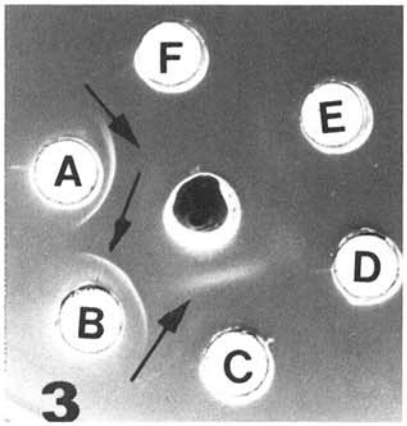

Fig. 3. Ouchterlony immunodiffusion test showing double bands on wells A and B containing mild TAV antigen, a single band adjacent to well C and E containing the type TAV antigen, and no band adjacent to wells D and F containing healthy spinach sap. The double bands adjacent to well A and B represent intact virions and protein subunits (arrows). The center well contained antisera prepared against the mild TAV strain; note the spur between well B and C (arrow) indicating a slight difference between the two strains.

TAV-M was present in high concentrations in spinach and tobacco leaves. Our initial attempts to purify the virus from spinach and tobacco leaves by the methods of Steere (15,16), Grogan et al (5), Lawson (10), and Marani (11) gave low yields (0.1–0.5 mg/100 g of leaf tissue) with low specific infectivity. TAV-M yields of 2–5 mg/100 g of leaf tissue were obtained from Bounty spinach (Fig. 1) following the method of Mossop et al (13). TAV-M yields of 0.5 mg/100 g of leaf tissue were obtained from *Nicotiana tabacum* (Xanthi NN) and preparations contained large amounts of host contaminants. Purified virus preparations remained infective for at least 15 days when stored at 4 C.

The ultraviolet absorption spectrum of purified TAV-M preparations was typical of nucleoprotein of a spherical plant virus with $A_{260/280} = 1.73$. Measurement of negatively stained preparations by electron microscopy showed that the normal diameter of the isometric viral particle was about 30 nm (Fig. 2).

Infectivity in crude sap was maintained for 2 days at 25 C. The virus was inactivated by 10 min of exposure at 65 C but not at 60 C; its dilution end point was between 10^{-4} and 10^{-5} from spinach sap and between 10^{-5} and 10^{-6} from tobacco sap.

Fig. 4. Comparison of (left) healthy tomato seedling and (right) seedling infected with mild TAV strain at the four-leaf stage. Note the dwarfing, impaired leaf development, and epinasty of older leaves on the infected seedling 30 days after infection.

TAV-M elicited a moderate immune response (microprecipitin titer of 1/512) in injected rabbits. A strong reaction was obtained in tube precipitation tests between virus antigen and homologous antibody (Table 1). A moderate reaction occurred between virus antigen and type TAV antibody, and a weak reaction occurred between TAV-M antigen and antibody to the Virginia strain of PSV (PSV 136). Immunodiffusion tests showed homologous reactions between TAV-M antigens and type TAV antisera (Fig. 3); no reaction occurred with PSV and CMV antisera tested.

Table 1. Reactions obtained in tube precipitation tests with mild tomato aspermy virus (TAV-M)[a] and antisera to five cucumoviruses[b]

	Reactions[c]					
Reciprocal dilution	**HA**	**TAV**	**PSV**	**PVAS 260**	**PVAS 242a**	**NS[d]**
1/64	+++	++	+	–	–	–
1/128	+++	+	–	–	–	–
1/256	++	+	–	–	–	–
1/512	+	–	–	–	–	–
1/1024	–	–	–	–	–	–

[a] Purified virus preparation of our mild TAV isolate (125 mg/ml).
[b] Antisera obtained from American Type Culture Collection, Rockville, MD.
[c] Antisera to the following viruses were tested: HA = virus isolate antiserum (ie, homologous antibodies), TAV 161 = type tomato aspermy virus antiserum, PSV 136 = peanut stunt virus antiserum prepared against the Virginia strain PSV, PVAS 260 = cucumber mosaic virus antiserum prepared against CMV-D, PVAS 242a = cucumber mosaic virus antiserum prepared against CMV-S, and NS = normal serum of rabbits before injection of virus protein.
[d] Normal serum served as control.

DISCUSSION

Host range studies showed that TAV-M has a host range nearly identical to that of type TAV described by Blencowe and Caldwell (2). Although the symptoms produced by TAV-M on most hosts studied were similar to those of type TAV, they were not as severe. Some major differences were apparent in host reactions. Type TAV did not cause local symptoms when inoculated on leaves of *C. sativus* and *D. stramonium* (2). TAV-M showed no evidence of local or systemic symptoms on cucumber leaves but caused local necrotic spots on cotyledons from which we were able to recover the virus. This result is in agreement with previous observations of type TAV reaction in cucumber (8). Other reports on several TAV isolates have shown that they produce local lesions (4) but not systemic symptoms on cucumber leaves (5,7).

TAV-M caused mosaic and leaf distortion in *D. stramonium*, which differs from previous observations of type TAV strains (2). Hollings (7) demonstrated that strains of TAV can be differentiated by symptom severity in tomatoes. In all tomato plants tested, TAV-M caused some dwarfing and leaf distortion (Fig. 4) but did not produce enations or seed aspermy in fruit typical of type TAV. Few TAV isolates have actually been recovered from tomato plants (2,17). Most reported aspermy viruses are derived from chrysanthemum and referred to by some authors as TAV (4,5,7,8,10,17). To our knowledge, TAV has not been isolated previously from tomato in Maryland. *A. hypogaea* and *Z. mays* are newly reported hosts for TAV.

Physical properties of TAV-M are within reported ranges for TAV (4) and cucumoviruses (19). Serological data provide the most useful means for establishing whether two virus isolates are related (1). Formation of precipitin lines noted in immunodiffusion tests between type TAV and TAV-M and the data obtained in tube precipitation tests indicate a close serological relationship exists between the two virus strains.

Serological differences indicate possible differences in coat protein structure or composition. Results of tube precipitation tests showed weak serological reactions between PSV (Virginia strain) and TAV-M that we were unable to detect in immunodiffusion tests. These reactions between TAV-M and PSV indicate a distant relationship between the two viruses. Previous studies have shown that such distant relationships occur between some aspermy type viruses and PSV (3,12).

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