A Strain of Eggplant Mosaic Virus Isolated from Naturally Infected Tobacco Plants in Brazil

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ABSTRACT

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A tymovirus was isolated from *Nicotiana tabacum* plants with mosaic symptoms from a commercial plantation in Brazil. The virus was readily sap-transmitted to *Chenopodium quinoa, C. amaranticolor,* and several species of the Solanaceae, and was transmitted experimentally by the chrysomelid beetle *Diabrotica speciosa*. Purified preparations contained isometric particles 26 nm in diameter that sedimented as two components with sedimentation coefficients of 52S and 108S. Electrophoretic analysis showed a single type of RNA of 2×10^6 Da and a capsid composed of a single polypeptide of about 22 kDa. The virus reacted with antisera to belladonna mottle, Andean potato latent, dulcamara mottle, and scrophularia mosaic viruses, and the type, Abelia latent, and tomato white necrosis (TWN) strains of eggplant mosaic virus (EMV). It was found to be serologically identical to TWN strain of EMV although it differed from this virus in some biological properties, i.e., its ability to systemically infect *Nicotiana tabacum* cultivars and its inability to cause systemic symptoms in tomato.

Tobacco (*Nicotiana tabacum* L.) is an important industrial crop in Brazil. Nearly 300,000 ha are cultivated annually, mostly in the southern states, which are responsible for 80% of the total production (1). About one third of the total leaf production is exported yielding an annual revenue of $500 million, while the rest is used by the local cigarette industry (28).

Among the diseases registered in tobacco in Brazil are several caused by viruses such as tobacco mosaic, cucumber mosaic, potato virus Y, Brazilian tobacco streak, alfalfa mosaic, tobacco leaf curl, and tomato spotted wilt (8,19). Recently, only tobacco mosaic and tomato spotted wilt viruses have caused some economic concern (G. Oliveira, personal communication).

During a routine disease survey of commercial tobacco plantations in Blumenau, state of Santa Catarina, plants exhibiting systemic mosaic symptoms were found. Subsequent studies demonstrated that the disease was caused by a tymovirus that is serologically related to several strains of eggplant mosaic virus (EMV). We report here the first description of the natural occurrence of a tymovirus in tobacco plants, and the virus will be referred to hereafter as EMV-T (EMV tobacco strain).

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MATERIAL AND METHODS

Virus isolate and maintenance. The virus was isolated from infected *N. tabacum* cv. Amarelinho plants collected from a commercial field in the state of Santa Catarina, Brazil. The isolate was passed through three single local lesion transfers on *N. tabacum* cv. TNN and maintained on the same host, which was also used as a test plant in all experiments.

Host range. Infected tobacco leaves were ground in 0.02 M neutral phosphate buffer containing 0.1% Na_2SO_3. The extract was applied with a cotton-tip swab onto the Carborundum-dusted leaves of a range of test plants.

Inoculated plants were kept in a greenhouse (25 to 30°C), observed for symptom development for 4 weeks, and then checked for virus presence by back inoculation on healthy *N. tabacum* plants.

Insect transmission. Chrysomelid beetles, *Diabrotica speciosa* Germ., were collected on squash and bean plants. They were allowed to feed for 48 h on healthy *N. tabacum* plants and then given a 48-h acquisition feeding period on systemically infected *N. tabacum* plants. Ten beetles were then transferred to each of 12 healthy plants for an inoculation feeding period of 48 h. Virus transmission was evaluated by symptom expression.

Purification. Isolate EMV-T was purified by the method described for mimosa mosaic virus (24). Infected tobacco leaves were harvested 12 to 15 days after inoculation and homogenized with 0.01 M phosphate buffer, pH 7.0, containing 0.1% Na_2SO_3 and 0.01 M EDTA. After clarification with 8% n-butanol, macromolecules were precipitated by adding 8% polyethylene glycol 6000. After stirring for 1 h, the solution was centrifuged at $6,000 \times g$ for 30 min. The pellet was resuspended in 0.01 M neutral phosphate buffer.

After a cycle of differential centrifugation ($6,000 \times g$ for 15 min and $180,000 \times g$ for 180 min), the partially purified preparation was layered on a sucrose gradient (0 to 40%) and centrifuged at $76,000 \times g$ for 150 min. The gradient was then monitored with an ISCO Model AU5 ultraviolet analyzer and fractionated by means of an ISCO Model 640 density gradient fractionator (ISCO, Lincoln, NE).

Serology. Specific EMV-T antiserum was raised in a rabbit by injecting intramuscularly 12 mg of purified virus emulsified with Freund's complete adjuvant, in 8 equal doses at 2 to 4 day intervals. About 25 days after the first injection and at weekly intervals thereafter, blood was collected from the marginal ear vein.

Double-diffusion test media contained 0.75% Noble agar (Difco, Detroit, MI) with 0.85% sodium chloride and 0.02% sodium azide. Antisera for comparative tests were kindly supplied by E. Luisoni (cucumber mosaic virus and squash mosaic virus), J. P. Fulton and H. A Scott (bean pod mottle virus), and R. Gamez (bean rugose mosaic virus). Antisera against the tymoviruses clitoria yellow vein virus (CYVV), Andean potato latent virus (APLV), physalis mosaic virus (PhyMV), poinsettia mosaic virus (PoMV), kennedya yellow mosaic virus (KYMV), turnip yellow mosaic virus (TYMV), desmodium yellow mottle virus (DeYMV), scrophularia mottle virus (ScrMV), belladonna mottle virus (BMV), okra mosaic virus (OkMV), cocoa yellow mosaic virus (CoYMV), dulcamara mottle virus (DMV), eggplant mosaic virus type strain (EMV-ts), and Abelia latent strain (EMV-Al) were from the Institut für Viruskrankheiten der Pflanzen, Braunschweig, Germany. Antisera against passionfruit yellow mosaic virus (PYMV), tomato white necrosis isolate of eggplant mosaic virus (EMV-TWN), and the comoviruses, Andean potato mottle virus and

cowpea severe mosaic virus, were produced at the Universidade de Brasilia, Brazil.

The antiserum to EMV-T was tested against EMV, APLV, ScrMV, DeYMV, ononis yellow mosaic virus (OYMV), and wild cucumber mosaic virus (WCMV) as antigens.

Electron microscopy. Leaf dip and purified preparations were stained with 1% sodium silicotungstate or 3% uranyl acetate and examined in a JEM 100C (Japan Electron Optical Ltd., Akishima, Japan) electron microscope. Particle measurements were made from micrographs of purified preparations of EMV-T with tobacco mosaic virus (TMV) as a particle size standard (34).

Protein electrophoresis. The molecular mass of EMV-T coat protein was determined by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (22). Low molecular mass protein standards (Sigma Chemical Co., St. Louis, MO) were run simultaneously with the coat protein of EMV-T to estimate molecular mass.

RNA electrophoresis. The RNA of EMV-T was extracted from purified preparations following the method of Aviv and Leder (3). Electrophoresis was in 1.5% agarose gel under denaturing conditions using glyoxal and dimethylsulfoxide (7, 23). Ribosomal RNA 28S and 18S from rat liver and TMV RNA were used as standards.

RESULTS

Host range and symptomatology. The virus was readily transmitted by mechanical means and infected mostly solanaceous hosts. Of 87 species tested in 25 families, only plants in the Amaranthaceae, Chenopodiaceae, and Solanaceae were susceptible to EMV-T (Table 1). Infected *N. tabacum* plants developed chlorotic and/or necrotic spots and necrotic rings 5 to 7 days after inoculation, followed by vein clearing, vein banding, and mosaic (Fig. 1). The following species were not infected by EMV-T: *Tetragonia expansa* Thunb.; *Celosia cristata* L.; *Catharantus roseus* L. (G. Dow); *Impatiens balsamina* L.; *Carica papaya* L.; *Beta vulgaris* L.; *Chenopodium murale* L.; *Rhoeo discolor* Hance; *Cichorium endivia* L. and *Cichorium intybus* L.; *Lactuca sativa* L.; *Tagetes erecta* L.; *Zinnia elegans* Jacq.; *Chrysanthemum* sp.; *Ipomea purpurea* (L.) Roth; *Brassica oleracea* L. var. *acephala* cv. Gongarsol and *Brassica alba* L.; *Raphanus sativus* L.; *Citrullus lanatus* (Thunb.) Mansf. cvs. Fairfax, Omaru Yamoto AG-214, and Crimson Sweet; *Cucumis sativus* L. cvs. Aodai Melhorado AG-191 and Híbrido Igarapé AG-196, *Cucumis melo* L. cv. Amarelo, and *Cucumis anguria* L.; *Cucurbita moschata* (Duchesne) Duchesne ex Poir. cv. Menina Brasileira and *Cucurbita maxima* Duchesne cv. Exposição; *Luffa cylindrica* M. Roem.; *Euphorbia heterophylla* L., *Ricinus communis* L.; *Manihot esculenta* Crantz; *Triticum aestivum* L. cvs. IAC-18, IAC-5, and BR-9; *Hordeum vulgare* L. cvs. T-7 and Antartica 4; *Zea mays* L. var. *saccharata* (Sturtev.) L. H. Bailey cvs. Super Doce and Doce Cristal; *Caesalpinia pulcherrima* (L.) Sw. and *Caesalpinia leyostachya* Ducke.; *Schilozobium parayba* Vell.; *Petrophorum dubium* Taub.; *Pterogyne nitens* Tul.; *Delonix regia* (Bojer ex Hook.) Raf.; *Pisum sativum* L. cv. Mikado; *Phaseolus vulgaris* L. cvs. Manteiga, Preto 123, Rico 23, Jalo, and Carioca; *Glycine max* (L.) Merr. cvs. Sucupira, Americana, IAC-8, and Tropical; *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* cv. Seridó; *Macroptilium atropurpureum* (Moc. & Sessé ex DC.) Urb.; *Centrosema pubescens* Benth.; *Allium cepa* L. cv. Pera do Rio Grande; *Hibiscus esculentus* L. cv. Chifre de Veado and *Hibiscus cannabinus* L.; *Malva parviflora* L.; *Sida* sp.; *Mirabilis jalapa* L.; *Oxalis* sp.; *Portulaca oleracea* L.; *Coffea arabica* L. cv. Novo Mundo; *Citrus* × *limonia* Osbeck.; *Antirrhinum majus* L.; *Capsicum annuum* L. cv. AG-10, *Capsicum baccatum* L., and *Capsicum frutescens* L.; *Solanum paniculatum* L. and *Solanum tuberosum* L. cvs. Aracy and Desireé; *Petroselinum sativum* Hoffman; and *Daucus carota* L. cvs. Brasília and Inverno.

Insect transmission and viral stability in sap. Transmission by *D. speciosa* occurred in two of the 12 tobacco plants tested.

The thermal inactivation point of EMV-T was between 80 and 85°C, and it was infective after a dilution of 10^{-9} but not of 10^{-10}. Sap was infectious for 19 days at room temperature and 300 days at 5°C. In leaves dried over silica gel and stored at –20°C the virus was infective for at least 300 days.

Purification. The virus was easily purified and yields ranged from 20 mg to 50 mg per 100 g of tissue. Virus preparations, centrifuged on sucrose density gradients, were separated into two distinct bands, 2.6 cm and 4.1 cm from the top of the gradient. Examination of negatively stained preparations in the electron microscope revealed that the top component contained mostly empty particles and the bottom component was mainly composed of full particles (Fig. 2). Sedimentation coefficients were calculated using the method of Brakke (6) with southern bean mosaic virus (32) as the standard. The top component had a sedimentation coefficient of 52S with a UV-light absorbing spectrum typical of protein with a peak at 274 nm. The bottom component had a sedimentation coefficient of 108S with a UV-absorbing peak at 260 nm, typical of nu-

Table 1. The response of host plants to the tobacco isolate of eggplant mosaic virus (EMV-T)

Host plant	Symptoms[a]	
	Local	Systemic
Amaranthaceae		
Gomphrena globosa L.	SI	–
Chenopodiaceae		
Chenopodium amaranticolor Coste & Reyn.	CS	–
C. quinoa Willd.	CS	SI
Solanaceae		
Datura metel L.	CS, NS	VC,M
D. stramonium L.	CS, NS	VC,M
Lycopersicon esculentum Mill. cvs. Kada, Santa Cruz, Angela Gigante, Capanema, LA.444-1	CS	SI
L. esculentum Mill. cv. Rey de los Tempranos and PI 732293-2V	CS, VC	SI
L. hirsutum H.B.K. PI 134417 and PI 127826	CS	SI
L. peruvianum (L.) Mill.	CS	SI
L. pimpinellifolium (L.) Mill.	CS	SI
Nicandra physalodes (L.) Gaertn.	SI	-
Nicotiana benthamiana Domin.	CS	VC,M,B
N. bigelovii (Torr.) S. Wats	CS	VC,M,CS
N. debney Domin.	CS	VC,M
N. glutinosa L.	CS	VC,M,N
N. occidentalis Wheeler	CS	CS,M
N. rustica L	CS	VC,M
N. sylvestris Speg.	CS	VC,M
N. tabacum L. cvs. Amarelinho, M 20, Kentucky 16, Judy's Pride, Xanthi-NC, Xanthi-NM, White Burley and H-425	CS	VC,VB,M
N. tabacum L. cvs. TNN, Turkish and Samsun	CS,NS,NR	VC,VN,VB,M
Petunia × *hybrida* Hort. Vilm.-Andr.	CS	M
Physalis floridana Rydberg	CS	M
Solanum nigrum L.	SI	SI
S. lycocarpum ST. Hil.	SI	SI
S. gilo Raddi cv. Verde Claro	CS	–
S. melongena L. cvs. Soxna, Imbú, Comprida Roxa, Black Beauty and Oriental Vegetable	CS	–

[a] CS = chlorotic spots, B = blisters, N = necrosis, NR = necrotic rings, NS = necrotic spots, M = mosaic, SI = symptomless infection, VB = vein banding, VC = vein clearing, VN = nein necrosis, – = not infected.

cleoprotein. The percentage of RNA present in the bottom component was estimated to be 35% according to Reichmann's procedure (26).

The extinction coefficient of purified virus, before component separation, was estimated to be 8.0 mg/ml/cm at 260 nm, with dry weight optical density data of four experiments, and this was the value used to calculate the virus concentration in purified preparations.

Serology. Antiserum to EMV-T, when tested in double-diffusion tests, did not react with healthy plant sap, and the highest titer obtained was 1:1,024 with infected plant sap or purified EMV-T preparations.

When tested in agar-gel double immunodiffusion, EMV-T reacted with antisera to APLV, BMV, DMV, PhyMV, ScrMV, and all strains of EMV tested but not with antisera to CYVV, PoMV, KYMV, TYMV, DeYMV, OkMV, CoMV, PYMV, or any cucumoviruses or comoviruses.

The antiserum to EMV-T gave positive reactions with EMV, APLV, BMV, DMV, and ScrMV.

Studies on the serological relationship between EMV-T and EMV-ts, EMV-AL, and EMV-TWN resulted in strong spur formation when EMV-T was tested in wells adjacent to EMV-ts and EMV-AL but not with EMV-TWN in all virus-antiserum combinations, indicating that the tobacco isolate was related, but not identical, to the former EMV strains and identical to the TWN strain. These results were confirmed by intragel adsorption tests and comparison of homologous and heterologous titers of antisera (data not shown).

Electron microscopy. Negatively stained leaf dip preparations of infected leaf tissue contained a large number of isometric particles both penetrated and unpenetrated by the stain, similar to those observed in purified preparations. The particles of EMV-T had a modal diameter of 26 nm.

Protein and RNA electrophoresis. In SDS-PAGE, EMV-T capsid was resolved as a single polypeptide with an estimated molecular mass of 22,000 Da (Fig. 3). Electrophoretic analysis resulted in a single RNA with an estimated molecular mass of 2 $\times 10^6$ Da. The extracted RNA was infective when inoculated on *N. tabacum* plants.

DISCUSSION

The tobacco isolate of EMV (EMV-T) has many properties in common with other viruses in the tymovirus group, especially with those of the EMV/APLV strain cluster. The virus strains belonging to this cluster differ serologically and in their pathogenicity for individual hosts (21).

The host range of EMV-T was essentially similar to those reported for several EMV and APLV strains. Like APLV-Caj and APLV-Hu (14), EMV-T infected tomato systemically without producing any evident symptom; however, other EMV strains cause systemic mosaic and an EMV isolate from Argentina causes a severe disease in this host (17).

Another difference between EMV-T and other EMV strains is that all *Nicotiana tabacum* cultivars inoculated with EMV-T developed systemic symptoms of vein clearing, vein banding, and mosaic. When inoculated in *Nicotiana tabacum,* EMV-ts caused symptomless infection only in the inoculated leaves (15); the Argentine tomato isolate induces chlorotic local lesions and occasionally systemic mosaic (17). Isolates EMV-Al (33), EMV-TWN (4), EMV-AR (12), and a *Solanum seaforthianum* isolate from Venezuela (13) do not infect *Nicotiana tabacum.* In eggplant, known EMV isolates become systemic, causing mosaic, except EMV-Al (33) and

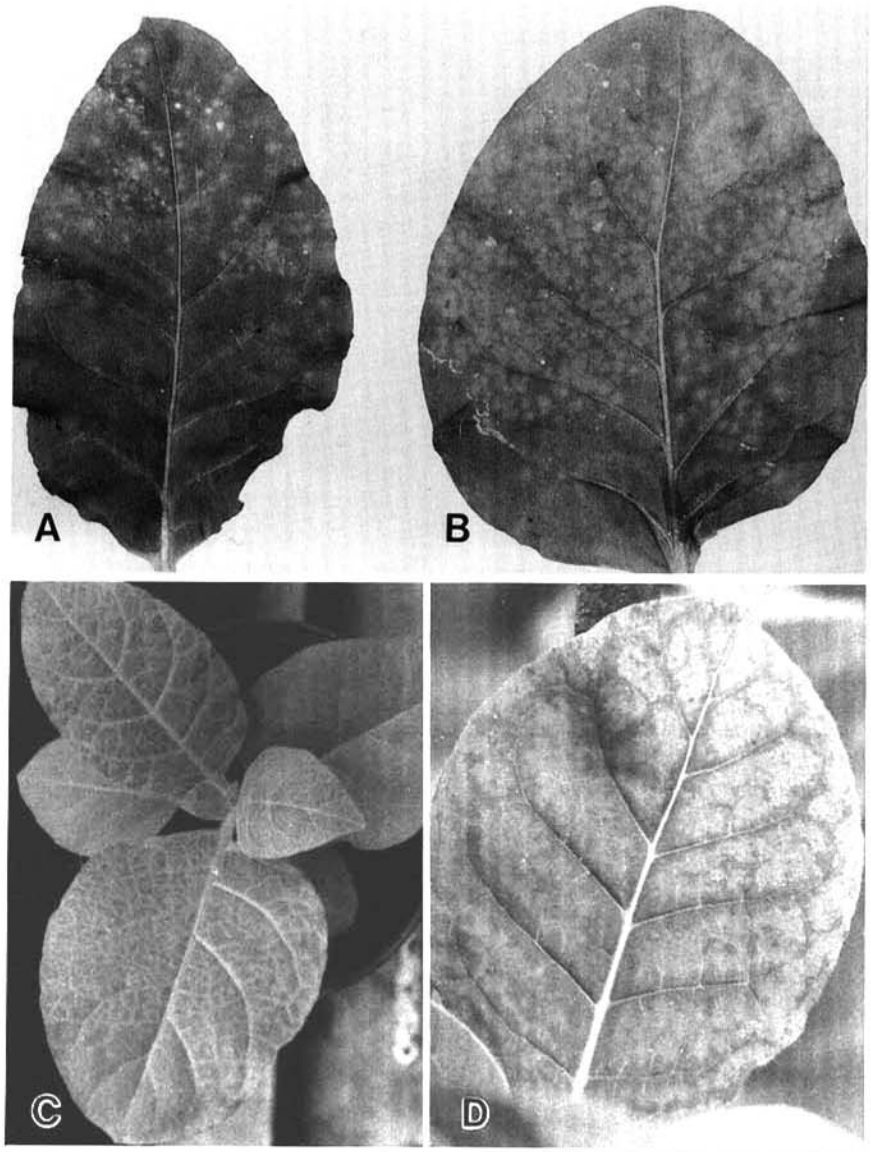

Fig. 1. Symptoms induced by eggplant mosaic virus tobacco strain (EMV-T) on *Nicotiana tabacum* cv. TNN leaves. **(A)** and **(B)** Chlorotic and necrotic spots and necrotic rings in inoculated leaves. **(C)** Systemic vein clearing. **(D)** Vein banding and mosaic.

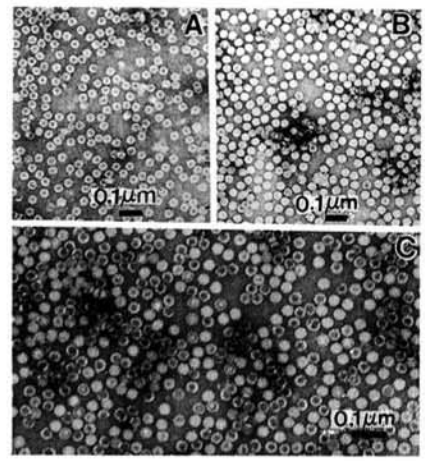

Fig. 2. Electron micrograph of purified preparations of eggplant mosaic virus tobacco strain (EMV-T) negatively stained with uranyl acetate. **(A)** Top component. **(B)** Bottom component. **(C)** Unfractioned preparation.

EMV-AR (12), which are systemic but produce symptomless infection. In this host, EMV-T induced only chlorotic local spots in mechanically inoculated leaves and could not be isolated from uninoculated tip leaves.

The estimated molecular mass of the coat protein (22,000 Da) agrees with that reported for EMV-TWN (4). Viral RNA has the size of most tymoviruses (2×10^6 Da), but the low molecular mass RNA that occurs in the top component of EMV (5), OkMV, and WCuMV (31) and in the bottom component of TYMV (20,27,31) was not detected in EMV-T.

There was a low level of transmission by *D. speciosa*. This chrysomelid beetle is polyphagous and has been reported as a vector for comoviruses (9,10,12,30), southern bean mosaic virus (29), tymovirus (11), carmovirus (2), and granadilla mosaic virus (25) in Brazil. This beetle could be the natural vector of EMV-T, since it, together with *Epitrix* spp. (G. Oliveira, personal communication), are some of the most important pests affecting the tobacco crop; *Epitrix* spp. could be considered as potential vectors since some are known vectors of other EMV strains (15–18).

Isolate EMV-T is the first strain of EMV isolated from naturally infected tobacco plants and differs biologically and serologically from type and Abelia latent strains, and biologically but not serologically from TWN isolate. Thus, it should be included in the APLV/EMV cluster in the serological classification system of tymoviruses (21).

At the moment, EMV-T is not of economic importance in Brazil because it was found just once in a restricted area and apparently does not interfere with plant productivity. Nevertheless, EMV-T may be considered a potential threat, since it could spread into other areas and give rise to more severe disease.

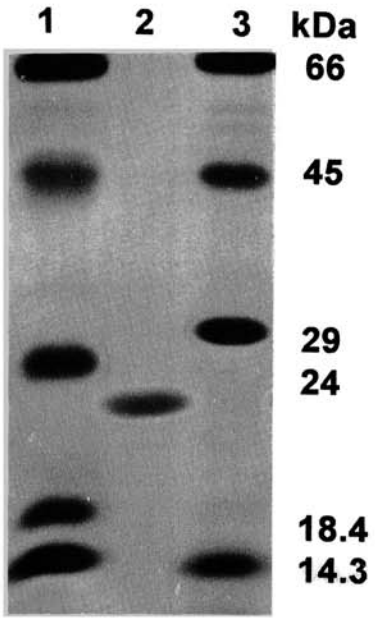

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the virion capsid protein of eggplant mosaic virus tobacco strain (EMV-T) (lane 2). Protein molecular mass markers (lanes 1 and 3).

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