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First Report of Tomato Mosaic Tobamovirus from Malaysia

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ABSTRAK

Satu penyakit mosaik di tomato telah dilihat di Cameron Highlands dan menunjukkan sejenis tobamovirus adalah penyebab berasaskan kepada morfologi zarah virus dan pengeluaran simptom di Lycopersicon esculentum *Mill. Virus telah dikenalpasti sebagai 'tomato mosaic tobamovirus' (ToMV) melalui ciri-ciri julat perumah dan serologi.*

ABSTRACT

A mosaic disease of tomato was observed in Cameron Highlands, Malaysia and a tobamovirus was implicated as the cause based on virus particle morphology and reproduction of symptoms in Lycopersicon esculentum *Mill. The virus was identified as tomato mosaic tobamovirus (ToMV) based on host range and serological properties.*

INTRODUCTION

Tomato has been grown on a considerable scale in scattered 1 – 2 hectare plots for more than twenty years in Cameron Highlands, Malaysia, a cool, highland area 1500 m above sea-level. In 1987, tomato plants showing virus-like symptoms including mild foliar mottling were observed for the first time. Virus infection of tomatoes had not previously been reported in Malaysia. Examination of leaf dip preparations showed that the tomatoes were infected with rigid rod-shaped particles. In this paper we present evidence that the virus is an isolate of tomato mosaic tobamovirus (ToMV).

MATERIALS AND METHODS

Virus Isolates and Maintenance

Tomato mosaic tobamovirus, Dahlemense strain ATCC PV394 (ToMV-394) and tobacco mosaic tobamovirus, common strain ATCC PV135 (TMV-135), were purchased from the American Type Culture Collection (ATCC, Rockville, MD). ToMV-394 was propagated in tomatoes, and TMV-135 in tobacco (*Nicotiana tabacum* cv. Speight G28).

Inoculation and Host Range

The virus from leaves of field-infected tomato cv. Local 828 showing mottling symptoms was mechanically transmitted to *Nicotiana glutinosa* L. Tomato leaves were macerated in a mortar with 10 mM phosphate buffer (pH 7.0) and rubbed on to 600-mesh carborundum-dusted leaves of test plants grown in a temperature-controlled room supplemented with cool white lights at 25°C. Limited host range studies were carried out with inoculations made using infected tomato or tobacco leaves as the inoculum source. Indicator plants inoculated included *Lycopersicon esculentum* Mill. cvs. Local 828, Grosse Lisse, *Nicotiana tabacum* L. cvs. Speight G28, Xanthi,

Kentucky 15, Burley 49, *N. rustica* L., *N. megalosiphon* Arg., *N. benthamiana* Domin., *N. glutinosa* × *N. clevelandii*, *N. occidentalis* Wheeler, *Datura stramonium* L., *Gomphrena globosa* L. and *Chenopodium amaranticolor* Coste & Reyn. Similar inoculation tests also were carried out with ToMV-394 and TMV-135.

Electron Microscopy

Leaf-dip preparations for electron microscopy were prepared from infected tomato foliage. Small pieces of tomato leaf material were crushed in drops of 2% phosphotungstic acid, pH 6.8. A small drop of the extract was then placed on carbon-strengthened, Formvar-coated 400-mesh copper grids and viewed in the Philips HMG 400 transmission electron microscope.

Purification

The viruses from tomato, ToMV-394 and TMV-135, were purified from leaves of tomato *L. esculentum* cv. Grosse Lisse or tobacco *N. tabacum* cv. Speight G28 by the polyethylene-glycol (PEG) precipitation method of Hollings and Huttinga (1976) with modifications. The infected leaves were homogenised in 50 mM phosphate buffer (pH 7.5) containing 125 mM Na_2SO_3 (2 ml buffer/g tissue). Chloroform was added (1 ml/g tissue) and mixed well. After clarification, polyethylene glycol (PEG 6,000) was added to the supernatant (4 g PEG/100ml supernatant). The precipitate was collected, resuspended, and followed by one cycle of differential centrifugation. The viruses were further purified on a 10-40% sucrose density gradient. The virus fraction, recovered from density gradients with a fractionator, was sedimented and resuspended in the same buffer.

Serology

Polyclonal antisera to ToMV from Malaysia, ToMV-394 and TMV-135, were produced in rabbits immunized using a series of two intravenous, one subcutaneous and one booster injection. For each rabbit a first injection of 250 µg/ml antigen was administered followed by a second injection of 500 µg/ml intravenously after one week. Four weeks later, 1 mg/ml of antigen emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously. Booster injections were given subcutaneously with 450 µg/ml of antigen emulsified with Freund's incomplete adjuvant one month later. The rabbits were bled at weekly intervals starting one week after the last injection. Antisera with a minimum reciprocal titer of 64 by gel immunodiffusion tests were used in the serological studies.

The direct double antibody-sandwich (DAS) protocol of Clark and Adams (1977) and the indirect ELISA protocol of Lommel *et al.* (1982) were used to compare the viruses. The immunoglobulin (IgG) was purified from antisera by ammonium sulphate precipitation and chromatographed on DEAE 52 cellulose (Whatman Biosystems Ltd.) on a 1 × 8-cm Bio-Rad Econolumn. For DAS-ELISA, the microtitration plates (polystyrene, flat-bottom) were coated with IgG (1 µg/ml), followed by purified virus samples at two-fold dilutions and incubated at 4°C overnight, and alkaline phosphatase-conjugated IgG applied and incubated at 37°C for 2 h.

For the indirect-ELISA similar microtitre plates were used and coated with purified virus samples at two-fold dilutions. After washing, antiviral antibody at 10 µg/ml was added and incubated for 3 h. Goat anti-rabbit IgG conjugate was applied and incubated for 2 h. The washing procedures were carried out using the Titertek Microplate Washer (Flow Laboratories Inc., Australia) and the absorbance values were measured with a Titertek Multiskan Plus

Reader (Flow Laboratories Inc., Australia) at 405 nm 30-60 min after the enzyme substrate was added.

Immunoelectron microscopy was performed as described by Milne and Luisoni (1977) and Hill (1984). Purified ToMV from Malaysia, ToMV-394 and TMV-135, at a concentration of 200 µg/ml with ToMV from Malaysia antiserum diluted at 1/100 with 10 mM phosphate buffer (pH 7.0) and incubated in a humid chamber at room temperature for 15 min. The grids were then examined in the transmission electron microscope for particle decoration.

RESULTS AND DISCUSSION

Host Reactions

The following host plants produced necrotic local lesions with the virus isolated from tomato: *N. glutinosa, N. tabacum* cvs. Xanthi, Speight G 28, Kentucky 15, *N. rustica, N. occidentalis, N. megalosiphon, N. glutinosa* × *N. clevelandii, D. stramonium, C. amaranticolor* and *G. globosa*. Similar reactions were observed when ToMV-394 was inoculated to the indicator plant species. Symptoms in *N. glutinosa, C. amaranticolor, N. rnegalosiphon, N. rustica, N. tabacum* cvs. Xanthi and Kentucky 15 showed similarity to that of TMV (Zaitlin and Israel 1975; Brunt 1986). However, ToMV could be further differentiated from TMV by its nonsystemic reaction in *D. stramonium, N. rustica* (Brunt 1986) *N. tabacum* cv. Kentucky 15, *N. megalosiphon* and systemic latent infection in *N. tabacum* cv Burley 49. Mild systemic mottling symptom was observed in *L. esculentum* cvs. Local 828, Grosse Lisse. The symptom in tomato cv. Local 828 was similar to the symptom observed in the field (*Fig. 1*).

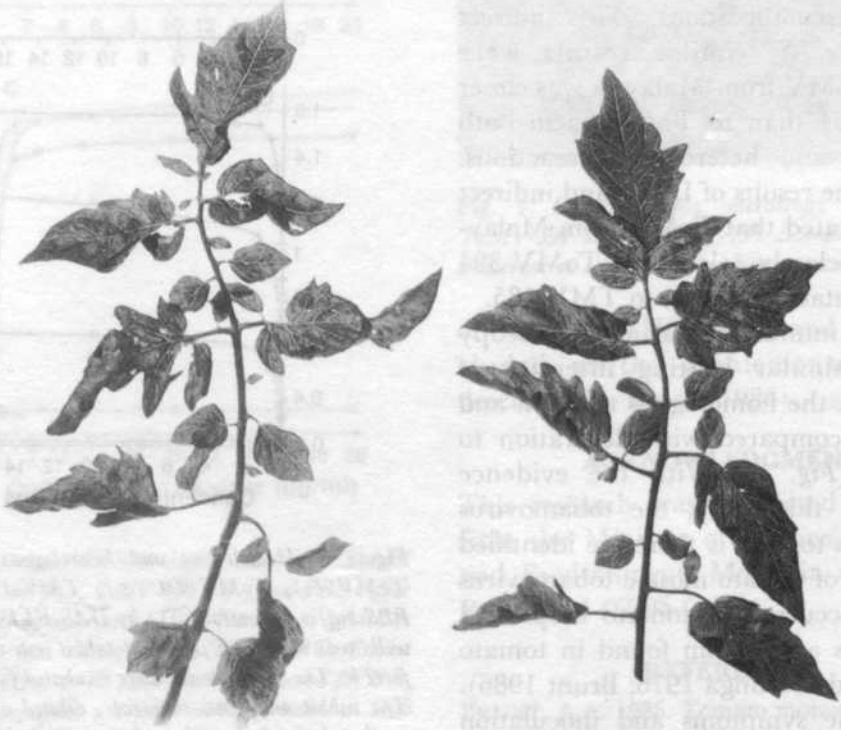

Fig 1: Field-infected (left) and inoculated (right) tomato with ToMV

Electron Microscopy

Field-infected tomato plants with mild mottling symptoms (*Fig. 1*) contained short rigid rod-shaped, virus-like particles. No virus particles were detected in symptomless plants. One UV-absorbing peak was observed on sucrose density gradients. Electron microscopic examination of the peak showed the same short, rigid, rod-shaped particles as those seen in leaf-dips. The modal particle length was 290 nm in purified preparations.

Serological Tests

When ToMV from Malaysia was allowed to react in both homologous and heterologous combinations in DAS-ELISA, it was clearly differentiated from ToMV-394 and TMV-135 (*Fig. 2*). The maximum reading was obtained in the homologous reaction. Similar reactions were also observed when ToMV-394 and TMV-135 antibodies were used in both homologous and reciprocal heterologous combinations. With indirect ELISA (*Fig. 3*) similar results were obtained; ToMV from Malaysia was closer to ToMV-394 than to TMV-135 in both homologous and heterologous reactions. Therefore, the results of DAS– and indirect ELISA indicated that ToMV from Malaysia is more closely related to ToMV-394 and more distantly related to TMV-135.

In the immunoelectron microscopy there was similar coating intensity of antibodies in the homologous reaction and ToMV-394 compared with decoration to TMV-135 (*Fig. 4*). With the evidence presented in this study, the tobamovirus isolated from tomato is therefore identified as an isolate of tomato mosaic tobamovirus because it occurred in tomato crops and TMV strains are seldom found in tomato (Hollings and Huttinga 1976; Brunt 1986). Based on the symptoms and inoculation studies in tomato cv. Local 828, the virus is the cause of the foliar symptoms in tomato

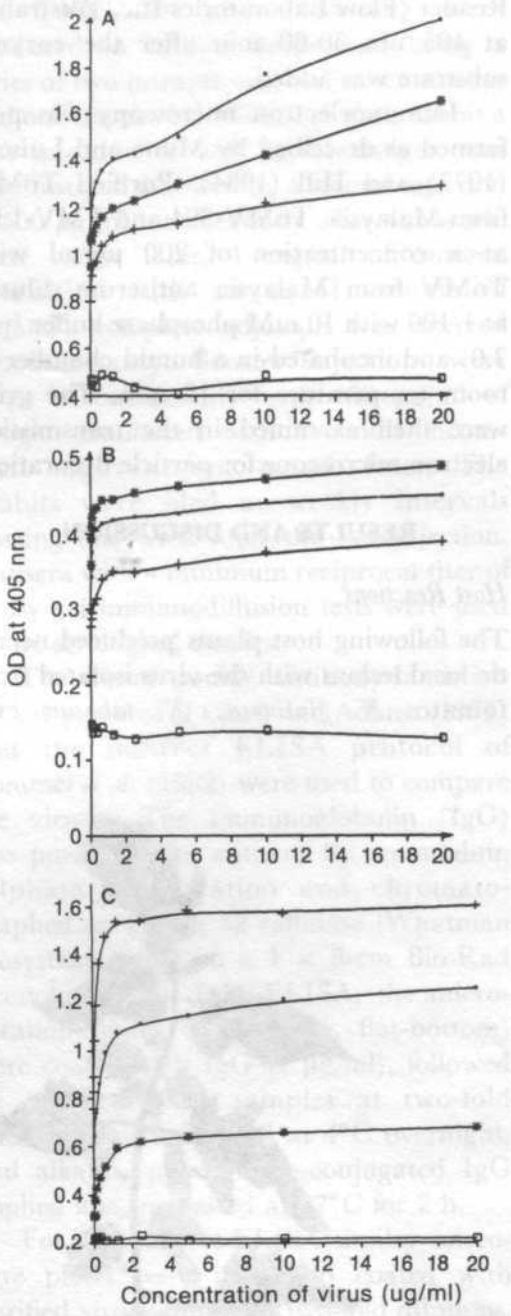

Figure 2: Homologous and heterologous reactions of ToMV (.), ToMV-394 (*), TMV-135 (+) and PBS buffer as control (□) by DAS-ELISA. Coating of wells was done with µg/ml of rabbit anti-virus globulins for 2 h. The viruses tested were incubated for 18 h at 4°C. The rabbit anti-virus conjugate, diluted at 1:3200 was incubated for 2 h. The substrate hydrolysis was 1 h. Antisera prepared against (A) ToMV, (B) ToMV-394 and (C) TMV-135.

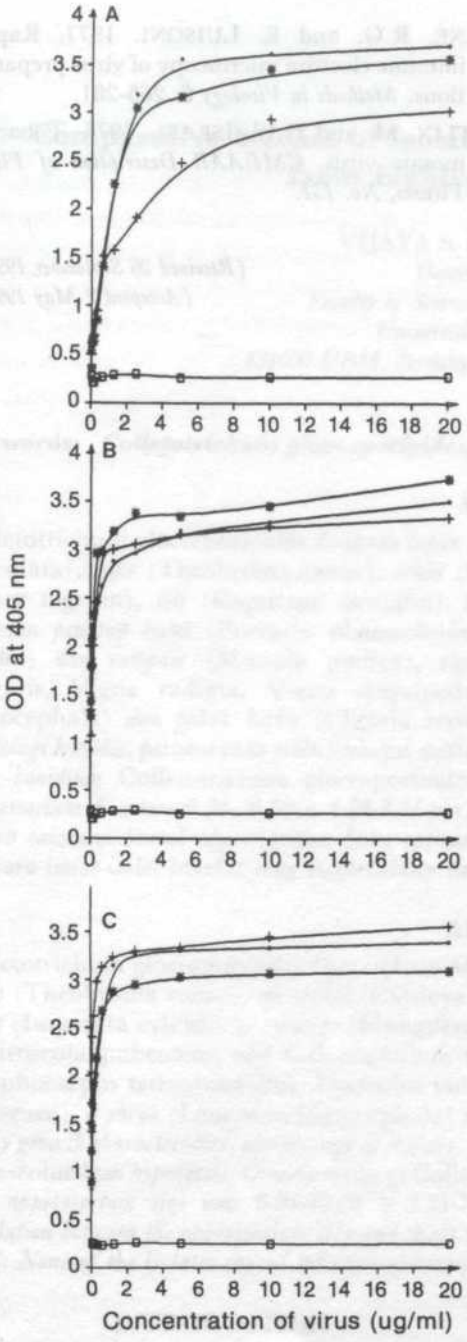

Fig. 3: Homologous and heterologous reaction of ToMV (.), ToMV-394 (*), TMV-135 (+) and PBS buffer as control (□) by indirect ELISA. The viruses diluted in coating buffer was incubated for 18 h at 4°C. Rabbit antivirus globulins at 10 µg/ml were incubated for 3 h, followed by conjugated goat anti-rabbit globulins at 1:00 dilution for 2 h. Substrate hydrolysis was 1 h. Antisera prepped against (A) ToMV, (B) ToMV-394 and (C) TMV- 135.

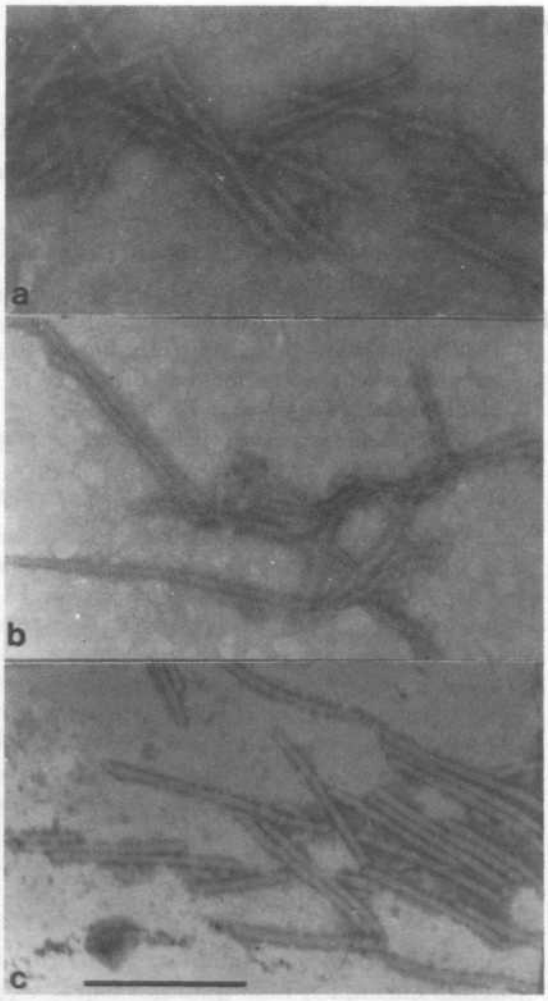

Fig. 4: Immunoelectron microscopy a. ToMV, b. ToMV-394 and c. TMV-135 decorated with ToMV antiserum at 1/100 dilution. Bar represents 300 nm.

on Cameron Highlands and can be included as the Dahlemense strain as described by Brunt (1986).

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