Electron Microscopic and Immunological Evidences that a Virus Infecting Tomato in Honduras is an Isolate of Tobacco Etch Virus*

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Abstract

Tomato plants with leaf deformation and mottling were found in commercial plantations in Honduras. Light microscopy demonstrated the presence of intranuclear crystalline inclusions and fibrous masses in the cytoplasm. Elongated, potyvirus-like particles were observed in negatively stained crude extracts. Ultrastructural examination confirmed the presence of intranuclear crystals as well as of lamellar inclusionts typical of infection by potyviruses. Symptomatology, cytopathology and particle morphology suggested tobacco etch virus (TEV) as possible causal agent. Anti-TEV serum reacted with extract from dried leaf samples from infected tomato plants. Further identification of TEV was made by *in situ* immunocytochemical studies using antisera against coat protein and cytoplasmic, laminar inclusions of TEV. Labeling by anti-TEV coat protein serum occurred on fibrilar particles scattered in the cytoplasm which must represent TEV virions *in situ*. Cytoplasmic inclusions, which occur as laminated aggregates and pinwheels were specifically labeled by anti-TEV cytoplasmic inclusion serum.

Key words: cytopathology, immunocytochemistry

During survey of a geminivirus-induced, yellow leaf curl-like disease, locally known as "colocha" in tomato (*Lycopersicon esculentum* Mill.) at Comayagua Valley, Honduras (7), plants with leaf deformation and mottling (Fig. 1) were noticed in several plantations, though in low incidence (less than 1%). Samples of these plants from 3 different plots were either dehydrated or fixed in a modified Karnovsky fixative solution (3% glutaraldehyde, 2% paraformaldehyde in 0.05M cacodylate buffer pH 7.2) for further identification under laboratory conditions in Brazil.

Epidermal strips observed under phase contrast light microscope revealed the presence of intranuclear crystals with square or triangular profile and fibrous mass in the cytoplasm (Fig. 2). Negatively stained leaf dip preparations of dried samples permitted the detection of elongated particles typical of potyviruses (Fig. 3). Thin sections of the leaf samples, further processed after post-osmication and embedding in Spurr medium and examined under transmission electron microscope, demonstrated the occurrence of prominent intranuclear dense crystals (Fig. 4) and large number of laminar aggregates and pinwheels inclusions (type 2 potyvirus inclusion [4]) in the cytoplasm (Figs. 4, 5) of most of the epidermal and mesophyl cells, thus confirming light microscopy findings. Thin, fibril-like particles were also seen scattered in the cytoplasm (Figs. 5). Eventually some chloroplast were seen with disorganized photosynthetic membrane system.

These observations, together with the field symptoms (8), strongly suggested that these tomato plants were infected by an isolate of tobacco etch virus (TEV), which is the only potyvirus infecting Solanaceae able to induce intranuclear, crystalline inclusions (5, 11). Additional biological and moelcular data on TEV may be seen in the review by Purcifull & Hiebert (11) and in the paper by Baunoch *et al.* (2). Outcherlony's double diffusion test with anti-TEV serum (kindly provided by Dr.D.E. Purcifull, of the University of Florida) using SDS-treated (10) dehydrated leaves as antigen gave positive reaction. Samples of symptomless tomato plants, collected as controls, did not contain the cytoplasmic or nuclear inclusions. No particle was detected in leaf dip preparations and serological tests were negative using these control samples.

To further study the TEV-infection process, an immunolabeling study was carried out in some tissue sections of infected tomato leaves, fixed only in the modified Karnovsky fixative and embedded in LRGold using a pro-

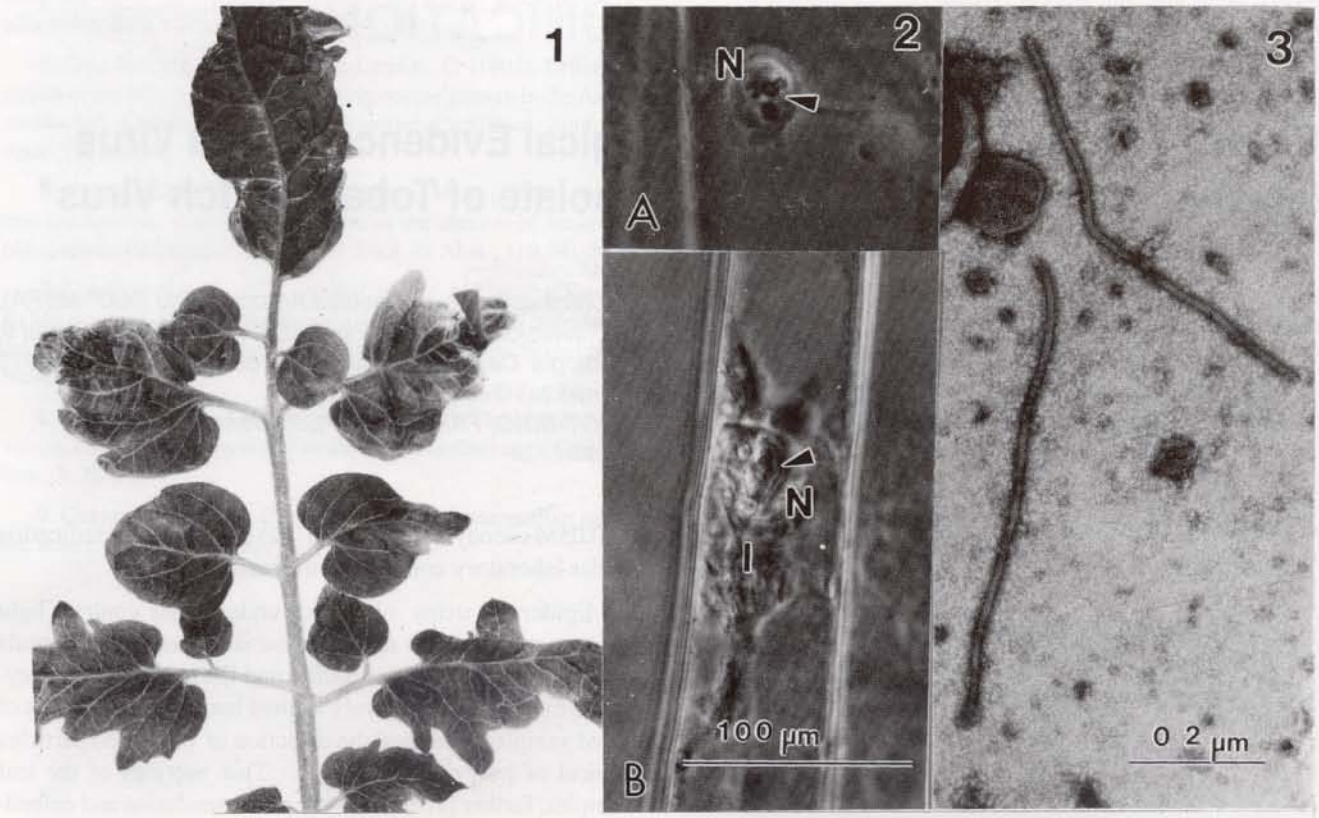
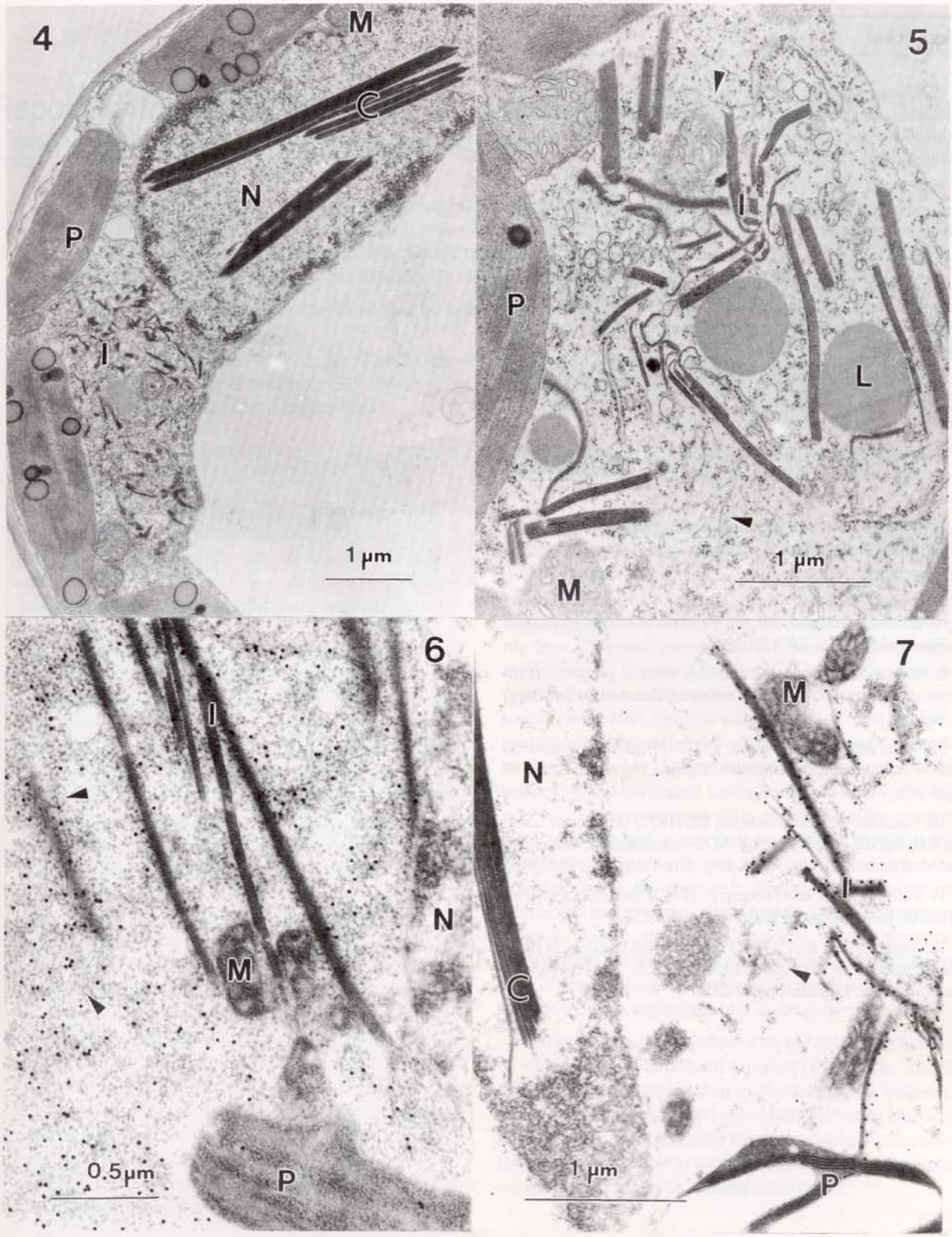


Fig. 1: *Leaf deformation and mottling in tomato plants grown at Comayagua Valley, Honduras.*
Figs. 2. A and B: *Phase contrast, light micrograph of epidermal strips of tomato leaves with mottling. Note the crystalline inclusion (arrowhead) in the nucleus (N). In B, a fibrous mass (I) can be seen adjacent to the nucleus.*
Fig. 3: *Elongated, flexuous particles present in uranyl acetate stained leaf dip preparation from affected tomato.*

gressive lower temperature protocol (12). Gold colored sections were treated either with anti-TEV coat protein and anti-TEV cytoplasmic inclusions sera (kindly provided by Dr. D.E. Purcifull, Univ. Florida), and indirectly labeled using protein-A colloidal gold (15 nm diameter). Thin fibril-like particles in the cytoplasm were consistently labeled by anti-TEV coat protein serum (Fig. 6) indicating that these particles must represent this Honduran isolate of TEV *in situ.* Anti-potato virus Y serum did not label the sections (figure not shown). Anti-TEV cytoplasmic inclusion serum specifically labeled the cytoplasmic laminar inclusions thus providing additional evidence that the potyvirus from Honduras is an isolate of TEV (Fig. 7). Anti-tobacco vein mottle cytoplasmic inclusion serum (a gift from Dr. T. Pirone, Univ. Kentucky) gave only a faint labeling (figure not shown) demonstrating that TVMV and TEV cylindrical inclusions, which are made up by a single polypeptide of 70 kDa (1, 11), share some common antigens as recently demonstrated by Hammond (6). The present results were basically similar to those reported with both aphid transmissible and non-transmissible isolates of TEV in the US (2, 3) Unfortunately it was not possible to obtain serum against intranuclear inclusion serum, thus labeling experiments with this structure were not carried out.

Because of the risks of introducing a virus not yet present in Brazil, only fixed or dried samples were brought in. Biological assays as transmission were not performed, but cytopathology and immunological studies both *in vitro* and *in situ* provided enough evidence to identify the virus found infecting tomato in Honduras as TEV, where there is no previous report of its presence. Though presently found in low incidence, TEV is easily dispersed by aphids and reported to cause yield losses in tomato (11) thus deserving continuous attention. It should be mentioned that there is a preliminary but yet unconfirmed occurrence of TEV in tomatoes in the state of São Paulo (9).



Figs 4-7: *Transmission electron micrographs of thin sections from mottling affected tomato leaves.*

Fig. 4: *Spongy parenchyma cell showing prominent crystalline inclusions (C) in the nucleus (N). Cytoplasmic laminar inclusions (I) are abundant in the area adjacent to the nucleus.*
Fig. 5: *Detail of the cytoplasmic inclusions (I) which appears as laminated aggregates, of the type 2 according to Edwardson's (4) classification of potyvirus-induced inclusions. Note thin, filamentous particles scattered in the cytoplasm (arrowheads).*
Fig. 6: *LRGold embedded tissue labeled with a serum against tobacco etch virus (TEV) coat protein. Gold particles are evident on filamentous particles (arrowheads) scattered in the cytoplasm but not on the laminated aggregates (I), chloroplast (P), mitochondria (M) or nucleus (N).*
Fig. 7: *Similar to fig. 6, but immunolabeled with anti-TEV cytoplasmic inclusion serum. An intense labeling is observed on the laminated aggregates (I). The remaining structures, including nucleus (N) containing a crystalline inclusion (C), chloroplast (P), mitochondria (M) and the thread-like structures (arrowheads) in the cytoplasm are not labeled..*

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