Ultrastructure of Potato Spindle Tuber Viroid-Infected Tomato Leaf Tissue

V. Hari

Department of Biological Sciences, Wayne State University, Detroit, MI 48202.
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ABSTRACT

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Tomato leaves from healthy and potato spindle tuber viroid (PSTV)-infected leaves were fixed and processed for transmission electron microscopy. The ultrastructure of the cells of infected leaves showed two important variations from cells of healthy leaves. First, (as in citrus exocortis viroid [CEV]-infected *Gynura* leaf cells) tomato leaf cells infected by PSTV developed the paramural bodies known as plasmalemmasomes. These plasmalemmasomes were of different sizes and shapes and showed internal structural variations. Second, infected leaf cells showed aberrations of the thylakoid membrane system of the chloroplasts and the grana were not well developed. No differences were found in the numbers of starch grains or electron-dense osmophilic granules in infected and healthy plants.

Additional key words: cytopathic vesicles.

Potato spindle tuber disease was shown to be caused by a unique type of pathogenic agent called a viroid and the specific pathogen involved was named potato spindle tuber viroid (PSTV) (1–3). Since then six other viroid-caused plant diseases (accepted viroid acronyms follow in parentheses) have been described: citrus exocortis (CEV), chrysanthemum stunt (CSV), chrysanthemum chlorotic mottle (ChCMV), cucumber pale fruit (CPFV), hop stunt (HSV), and the cadang cadang disease of coconuts. The pathogenic agent is a low-molecular-weight RNA with highly specialized structural features and (at least in the case of PSTV) the viroid RNA structure has been shown to be made of a closed circle composed of 359 nucleotides (5).

The primary symptom induced by all the viroids is the extreme stunting of the infected plants. Other symptom similarities of viroid infection include chlorosis and blistering of the leaves, but the severity of the disease symptoms varies with the individual viroid strain. It has been reported (8) that the primary internal cytopathic effect in *Gynura aurantiaca* plants infected by CEV is the production of enlarged invaginations of plasma membranes known as plasmalemmasomes (8). However, in a recent report (7) plasmalemmasomes were reported in healthy plants; the authors stated that the real cytopathic change is not the presence or absence of plasmalemmasomes but that these are highly distorted in the infected cells. In the same study it was found that in a different host plant, tomato, the main cytopathic change caused by a different viroid, namely CPFV, is the accumulation of electron dense material in the chloroplasts resulting in displacement of the thylakoid membranes to the periphery of the chloroplast. Such a chloroplast abnormality also was seen in CPFV-infected *Gynura*. Thus, the types of cytopathic changes vary depending on the host plant and viroid.

There has been no report on the ultrastructural changes caused by PSTV. This paper describes the ultrastructural cytopathological changes in tomato leaves following infection by PSTV.

MATERIALS AND METHODS

Inoculum and tissue source. The original inoculum of PSTV was obtained from H. Murakishi of the Dept. of Plant Pathology, Michigan State University, Lansing, MI and is the same as the original Beltsville isolate (4). Tissue material for electron microscopy was obtained from tomato plants (*Lycopersicon esculentum* 'Rutgers') which had been inoculated mechanically by a tissue homogenate prepared from PSTV-infected leaves (3). External symptoms of the infection appeared about 15–20 days post inoculation. The inoculated plants were stunted, the leaves showed epinasty and other typical symptoms of PSTV infection (3).

Tissue processing and electron microscopy. Disks 1 mm in diameter were cut from five different tomato leaflets showing typical external symptoms of PSTV infection. Tissue extracts were prepared from the remnants of the infected leaflets from which disks had been cut and the extracts were bioassayed for presence of viroid by inoculating leaves of the local lesion host *Scopolea sinensis*. In all cases the samples selected produced many pin-sized lesions typical of viroid infection (9). The selected tissue samples were fixed and processed for electron microscopy (6) as follows. The samples were fixed for 4 hr in 0.08 M cacodylate buffer, pH 7.4, containing 5% glutaraldehyde and 4% paraformaldehyde. The fixed specimens were washed three times at half-hour intervals with 0.1 M cacodylate buffer, pH 7.4, containing 3% sucrose. The samples were postfixed in 1% osmium tetroxide dissolved in a solution of 0.1 M cacodylate buffer and 2% sucrose. Following three half-hour washes in 0.1 M cacodylate-3% sucrose, pH 7.4, the samples were dehydrated in an alcohol series, sequentially followed by propylene oxide, proplylene oxide plus epoxy resin (1:1,v/v), and finally embedded in epoxy resin (6). Thin sections were cut from selected pieces of the embedded tissue, stained with uranyl acetate and lead citrate, and viewed with a Philips EM 300 electron microscope.

RESULTS

Ultrastructural effects of PSTV infections. Two cytopathic membrane system differences were immediately apparent in PSTV-infected tissues. The first was the presence of many cytoplasmic vesicles formed by invaginations of the plasma membrane (Fig. 1A) or of the tonoplast (Fig. 1B). These paramural bodies were of different sizes (Fig. 1A–C), contained multiple membrane-enclosed chambers (Fig. 1C), and were not found in uninfected cells.

The second major cytopathic change noted was the abnormal appearance of the grana and thylakoid membranes of the chloroplast. In a normal cell the thylakoids are uniformly stacked up at intervals to form the grana (Fig. 2A), but in the PSTV infected cells the thylakoid does not stack up uniformly and appears to be distorted (Fig. 2B). The abnormality associated with the thylakoids does not appear to be due to the presence of electron-dense granules and starch grains because these also are found in the chloroplasts of

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uninfected cells with no apparent membrane system impairment (Fig. 2A).

No differences were observed in the numbers of starch grains, and electron-dense material between healthy controls and PSTV-infected leaves. Occasionally, other types of membrane structures which resembled coiled watch springs were noticed (Fig. 1D); these also have been noticed in tobacco etch virus-infected cells, (V. Hari, *unpublished*).

DISCUSSION

Infection of tomato plants by PSTV causes externally recognizable symptoms such as stunting, leaf curling, and chlorosis. The only cellular abnormality described so far is the presence of hypertrophied nucleii in infected cells (2), but detailed ultrastructural studies have not been reported. The present investigation showed that the main cytopathic changes found in PSTV infected leaf cells were the presence of large numbers of paramural bodies, and the abnormal development of the chloroplast membrane organization.

Paramural bodies of the type reported here also have been seen in citrus exocortis viroid-infected, but not in healthy, leaves of *Gynura* (8). This has been disputed recently in a report (7) showing that paramural bodies also are found in healthy *Gynura* plants, although there is a qualitative difference in that the paramural bodies found in the CEV-infected *Gynura* were distorted (7). The

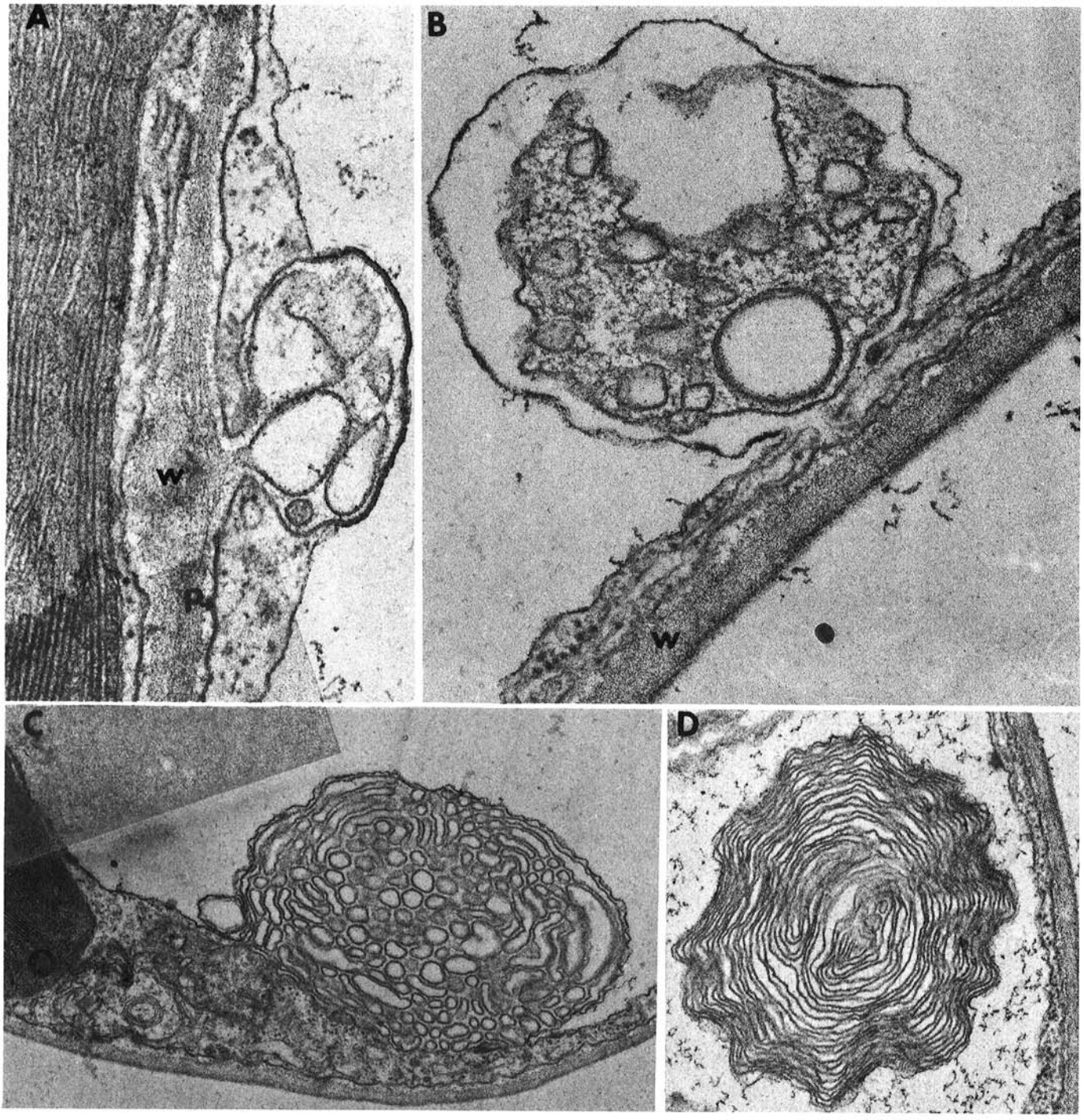

Fig. 1. Paramural bodies and membrane abnormalities in potato spindle tuber viroid-infected leaf cells. **A,** plasmalemmasome formed by invagination of plasmamembrane into vacuole; **B,** similar body formed by tonoplast; **C,** multiple vesiculated paramural body; **D,** watchspring-like multilayered membrane body found in cytoplasm. W=cell wall; p=plasmamembrane. A–C, ×70,000; D, ×38,000.

reasons for the disparity in the two investigations is not clear. Paramural bodies of the type found in PSTV-infected plants were not observed in any of the uninfected tomato samples in the present study.

Abnormal chloroplasts have been noted in cucumber pale fruit viroid (CPFV) infected tomatoes but not in *Gynura* plants infected by this viroid. In the tomato plants infected by CPFV, the thylakoid membranes were found to be pushed to the periphery because of the presence of electron-dense granules, and moreover, the number of starch grains was found to be less than in the control plants (7). The data presented here show that in tomato plants infected by PSTV there are no differences in the numbers of starch grains or of electron-dense material in the infected cells compared to the healthy. The main difference noted here was the poor stacking of the grana and the presence of loosely arranged thylakoid membranes. The lack of a compact thylakoid system, apparently has nothing to do with the presence of the electron dense material since healthy leaf chloroplasts which also have similar amounts of electron dense material are unaffected.

Overall, it appears that infection of plants by PSTV and other viroids involves the membrane system of the plant cell (8). These changes may simply be the result of the type of biological stress imposed on specific plants by the viroids or they may have implications relating to the sites of activity in the viroid replication cycle.

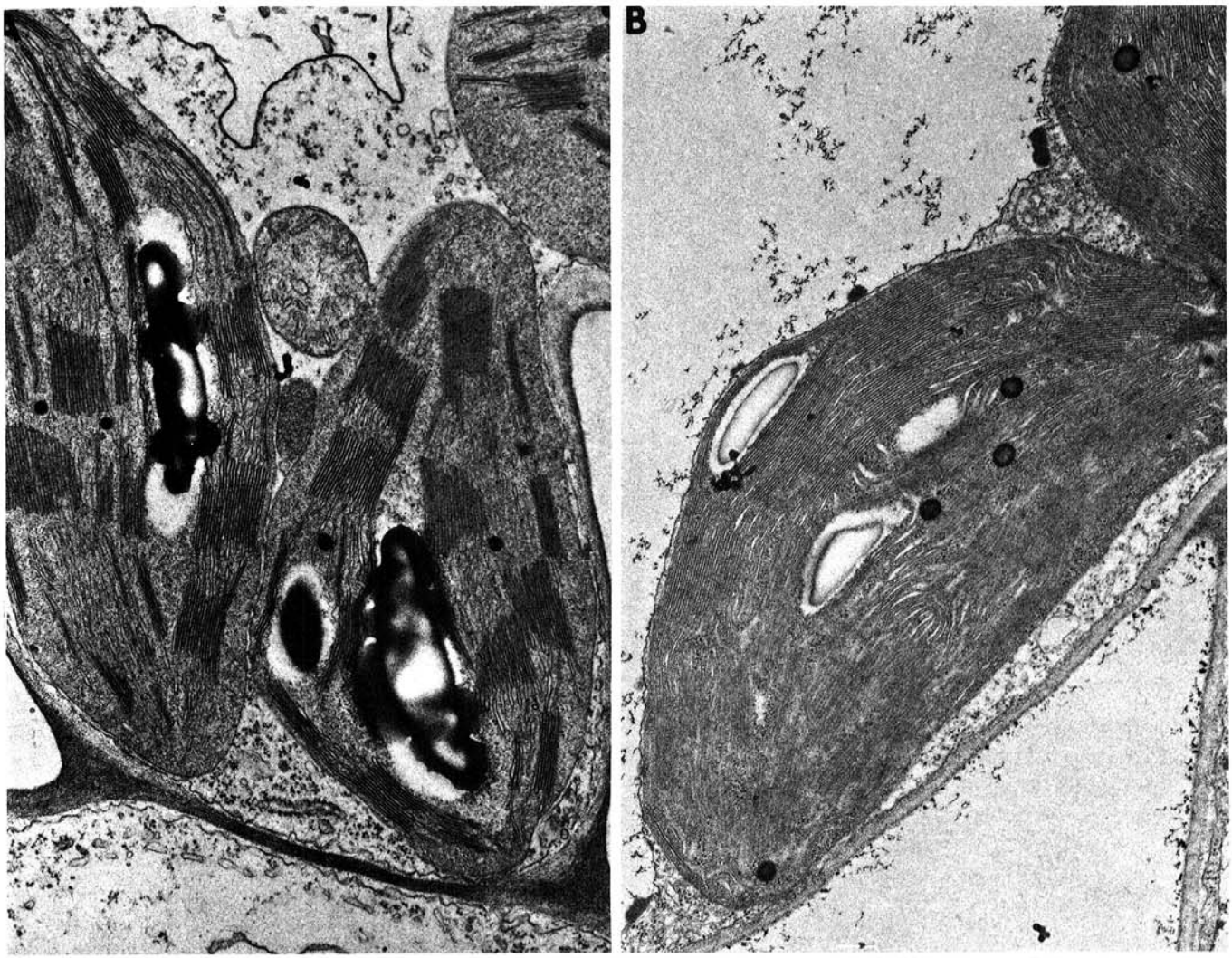

Fig. 2. Chloroplasts of **A**, healthy and **B**, potato spindle tuber viroid-infected tomato leaves. Note the starch grains and electron-dense globular bodies in both healthy and infected cells. The chloroplasts from healthy leaf cells show well formed grana (A) whereas the chloroplasts of infected cells show almost no grana and thylakoids are very loosely arranged (B). Magnifications both ×22,000.

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