CYTOPATHIC CHANGES IN VIROID-INFECTED LEAF TISSUES[1)]

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Introduction

Eight different viroids have been described so far as a causal agent of plant diseases since potato spindle tuber disease was confirmed to be caused by a viroid, a novel pathogen[4)]. Recently, the ultrastructural changes caused by viroid infections have been studied by several workers[2,3,6,7,8,11,13,14)] but their results are not always coincident at present. SEMANCIK and VANDERWOUDE[11)] first pointed out that citrus exocortis viroid(CEV) induced abundant production of paramural bodies (plasmalemasomes), cytopathic effects at the plasma membrane in *Gynura aurantiaca*. In contrast to this finding, using the same system, WAHN *et al.*[13,14)] reported that the actual cytopathic changes were aberrations of the cell walls and of the plasmalemasomes themselves. HARI[6)] also reported on ultrastructural changes of tomato leaf tissues infected with potato spindle tuber viroid(PSTV) showing production of paramural bodies and aberrations of the thylakoid membrane systems of the chloroplasts.

To determine which cytopathic effects occurred in each host-viroid combination, we conducted some comparative experiments using cucumber plants infected with hop stunt viroid(HSV) and cucumber pale fruit viroid (CPFV) and tomato plants infected with PSTV, CEV, HSV and CPFV, respectively.

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Materials and Methods

Viroids and plants

Viroids used in this experiment were HSV[10], CPFV* (from Dr. SÄNGER, Max-Planck-Institut, W. GERMANY), PSTV* (from Dr. SÄNGER) and CEV* (from Dr. SÄNGER). They were propagated in tomato (*Lycopersicon esculentum* cv. Rentita) and cucumber (*Cucumis sativus* cv. Suyo) plants. Inoculated plants were grown in a greenhouse at 28 C[10].

Electron microscopy

Apical leaves of each plant were cut off 5 weeks after inoculation. Fixation and embedding procedures were the same as those described in a previous report[12]. Ultrathin sections were cut by an ultramicrotome (Reihelt-Jung OmU4) equiped with a diamond knife. Specimens were viewed by an electron microscope (JEM 100 B, 80 kV) after double staining by uranyl acetate and lead citrate.

Results and Discussion

Cytopathic changes in cucumber plants infected with HSV and CPFV

Symptoms caused by HSV were quite similar to those caused by CPFV in cucumber plants. Each cucumber plant infected with HSV and CPFV showed very severe symptoms such as yellowing and shortening of internodes[9]. Table 1 summarized the cytopathic changes caused by both viroids in the infected cucumber plants and indicated that their internal symptoms were almost identical. One of the typical alterations is aberration of cell

TABLE 1. Cytopathic changes in cucumber and tomato plants infected by viroids

Host	Cucumber (Suyo)			Tomato (Rentita)				
Viroid	HSV	CPFV	control†	HSV	CPFV	PSTV	CEV	control†
External symptom	++	++	−	−	−	++	+++	−
Papamural body	+	+	+	+	+	+	+	+
Aberration of cell wall	++	++	−	−	−	++	++	−
Chloroplast disintegration	++	++	−	+	+	+++	+++	−
Disappearance of tonoplast	+	+	−	+	+	+	+	−

† healthy plants

* CPFV, PSTV, CEV, were imported with Quarantine permission No. 55 Yokoshoku Dai 1035 gou.

walls in all tissues, such as epidermis, palisade parenchyma, spongy parenchyma and vascular bundles. As shown in Plate I, the cell walls of HSV and CPFV-infected cucumber leaves are extremely distorted. These areas were characterized by cell walls with corrugated profile and irregular thickness. These aberrations appear to lead to a structural deformation of the cells themselves, resulting in external symptoms. Another alteration in the infected leaf cells was disintegration of the chloroplasts (Plate II). Furthermore, these cells having disintegrated chloroplasts usually accompanied with breakdown of tonoplast and production of granules within the cytoplasm. These structural changes are almost the same as those of tomato plants infected with PSTV and CEV (Plate III and IV).

Cytopathic changes in tomato plants infected with HSV and CPFV

Tomato plants showed no symptoms when infected with HSV or CPFV, but was proved to be capable of carrying these agents[9]. Therefore, it was rather difficult to find out distinctive alterations of leaf cells in the infected tomato plants. Disintegration of chloroplasts, however, sometimes (not often) occurred in these systems.

Cytopathic changes in tomato plants infected with PSTV and CEV

Symptoms caused by CEV were similar to those caused by PSTV in tomato plants, and were very severe in both cases. Cytopathic changes caused by both viroid infections were also almost the same (Table 1). Aberrations of cell walls such as corrugated profile and irregular thickness in tomato plants infected by PSTV and CEV were frequently observed. In addition, characteristic alterations of the chloroplasts within mesophyl cells were also frequently found (Plate III and IV).

Sequencial events of chloroplast disintegration by viroid infections

Disintegration process of the chloroplasts in tomato palisade and spongy parenchymatous mesophyl cells infected by PSTV and CEV has some similarities in cucumber plants as mentioned above. They are as follows ; ① swelling of the chloroplasts, ② abserration of their thylakoid membranes system, ③ distortion of their grana, ④ irregular arrangement of their stroma lamellar, ⑤ breakdown of their en-

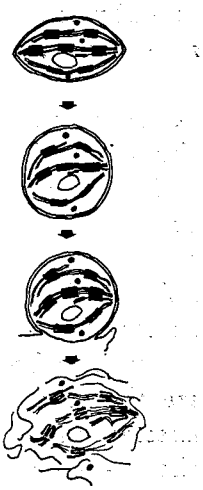

Fig. 1. Schematic presentation of chloroplast disintegration procedure in association with viroid infections.

velope, and ⑥ release of their inner contents into cytoplasm (Fig. 1). However, no differences were observed in the size and number of starch grains and plastoglobules (osmiophilic globules) in the infected and healthy plants (Plate II, III and IV).

Since no aberration of membrane systems of the chloroplasts was found in healthy plants (Plate II A and B), and the mesophyl cells adjacent to the diseased cells showing chloroplast aberrations showed no cytopathic changes having intact chloroplasts (Plate III, left), we presumed that such aberrations of membrane systems of the chloroplasts were one of the cytopathic effects in viroid infections. There are some similarities between these cytopathic changes and those caused by some plant virus infections[1,5].

Other cytopathic changes in viroid-infected plants

In healthy mature mesophyl cells, cytoplasm containing cell organelles is usually located at the periphery of cell walls, and vacuoles limitted by tonoplast are located in the middle of the cells (Plate II A and B). On the other hand, in the viroid-infected plants, other cytopathic changes such as disappearance of central vacuole and plasmolysis were also frequently found (Plate III and IV). These denatured cells were vertically distributed from palisade to spongy parenchymatous cells to form a denatured cell zone (Fig. 2).

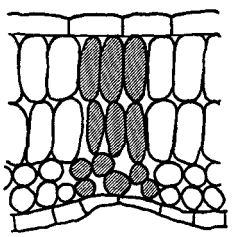

Fig. 2. Schematic presentation of a denatured cell zone in a viroid-infected plant, in which some denatured cells (oblique lined) were vertically distributed from palisade to spongy parenchymatous cells.

On the basis of our observations, cytopathic changes in viroid-infected plants, such as aberrations of cell walls and distortions of chloroplast membranes, in association with their external symptoms, were frequently found in both cucumber-HSV and-CPFV, and tomato-PSTV and-CEV systems, but indistinctive in tomato-HSV and-CPFV symptomless system.

It is unlikely that paramural bodies[3,6,11] are the primary cytopathic effects in viroid-infected plants because these structures are also present in healthy plants. It is also rather difficult to conclude from our investigation that "the viroid specific cytopathic changes are alterations of the paramural bodies themselves" as mentioned by WAHN *et al.*[13,14]

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Explanation of Plate

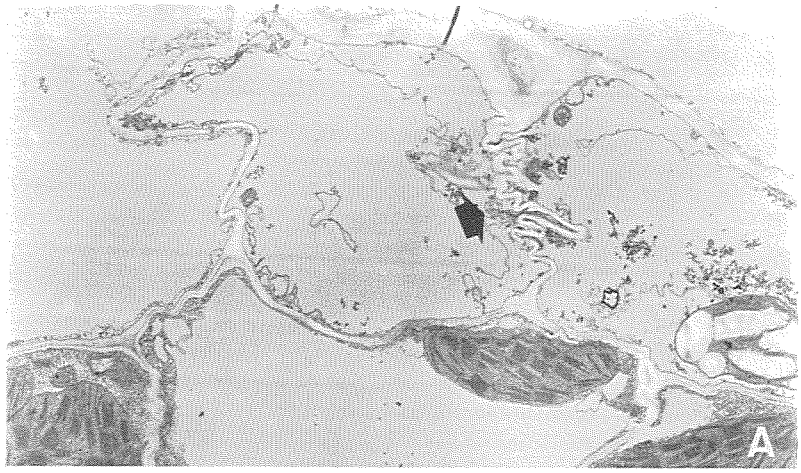
Plate I

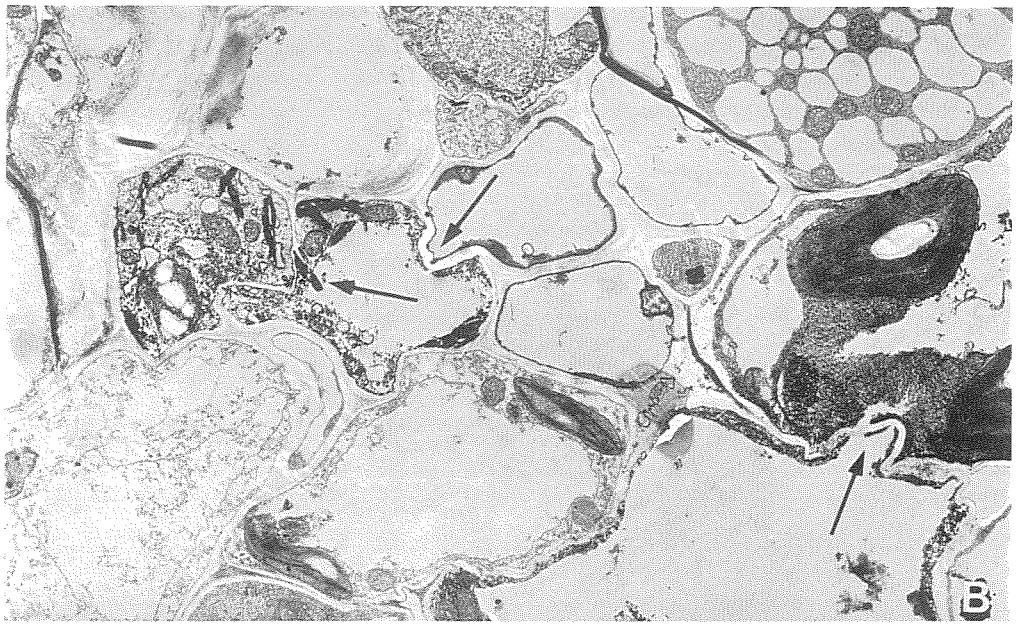
Aberrations (arrows) of cell walls of cucumber plants infected with CPFV (A and B) and HSV (C).

Photo A. upper epidermis. ×6,400

Photo B. vascular bandles. ×6,400

Photo C. lower epidermis. ×6,400


A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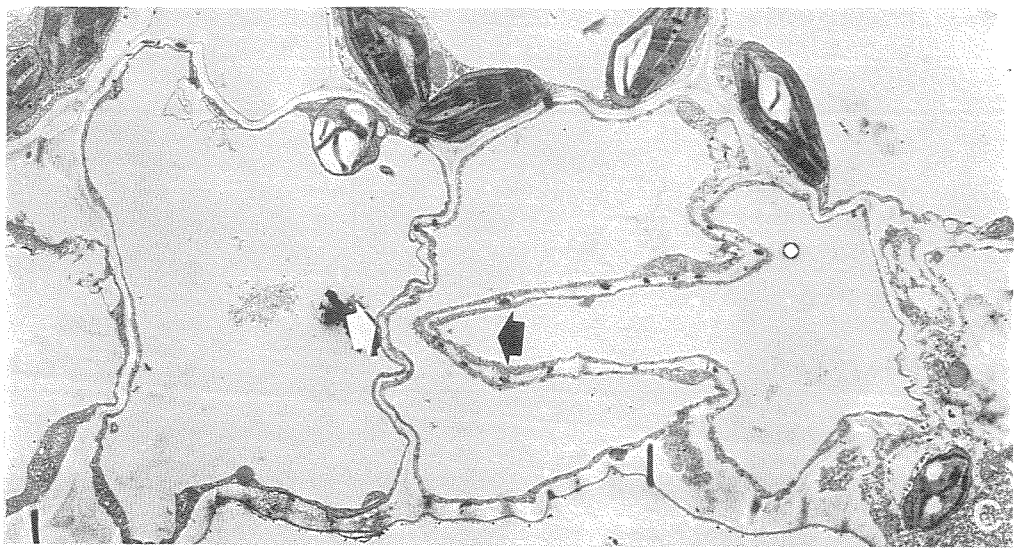

Plate II

Disintegation of chloroplasts in plants associated with viroid infections.

Photos A, B. Ultrathin sections of palisade (A) and spongy (B) parenchymatous mesophyl cells of healthy cucumber plants. ×6,000
N: nucleus; C: chloroplast

Photo C. A palisade parenchymatous cell of a cucumber plant infected by HSV. Note disappearance of the envelopes and aberrations of thylakoid membrane systems of chloroplasts. ×6,000

Photo D. A spongy parenchymatous cell of a tomato plant infected by CEV. Note various stages of chloroplast disintegration. ×6,000
double arrow: double layers; single arrow: single layer; star: no layers; white arrow: plastoglobule

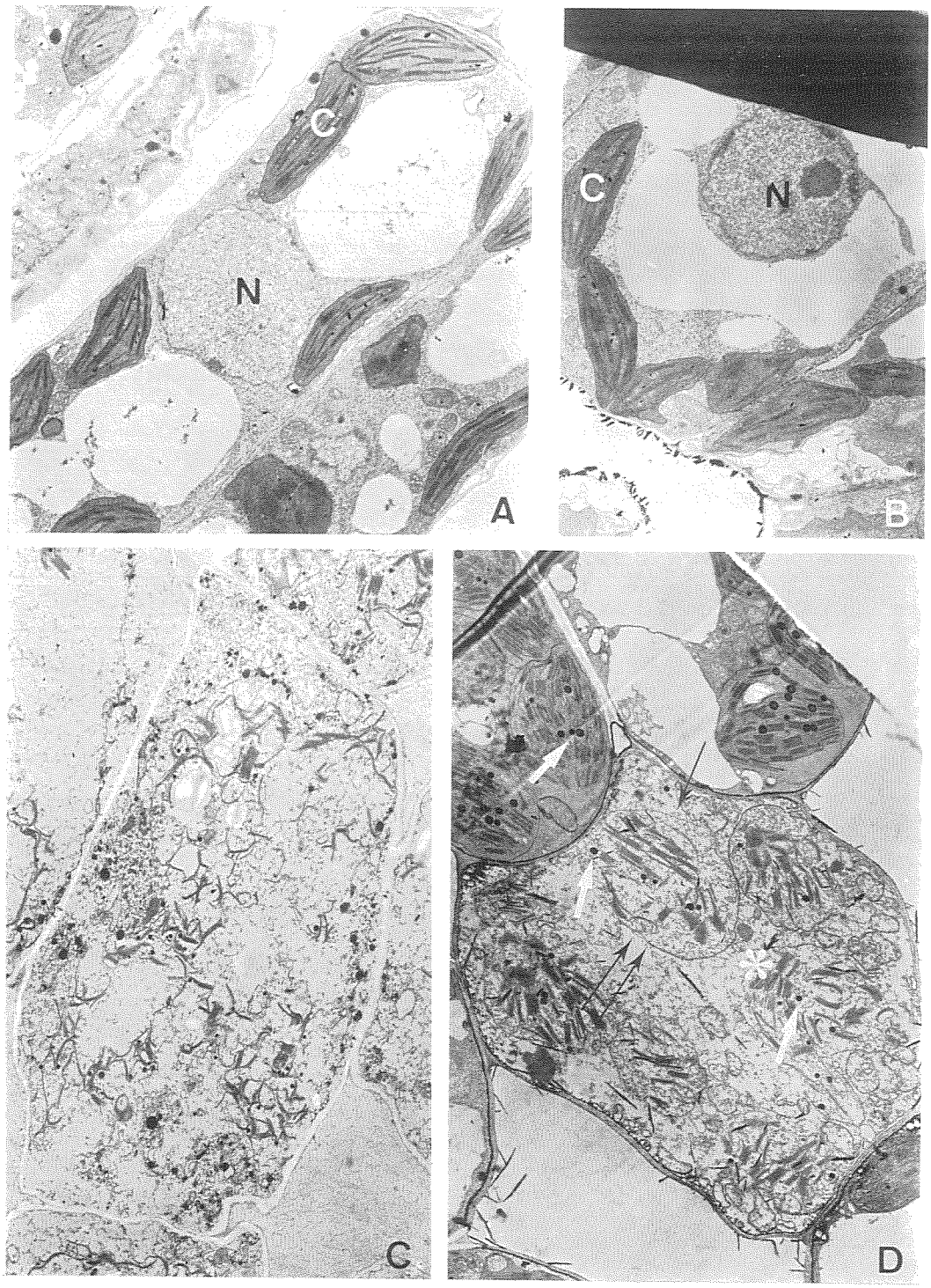
C
N
A
C
N
B
C
D

Plate III

Cytopathic changes in the palisade parenchymatous cells of a tomato plant infected by CEV. Note a cell (left) in which cell organelles are normally arranged and two neighbouring affected cells showed disappearance of tonoplast and breakdown of chloroplast envelope. ×6,400

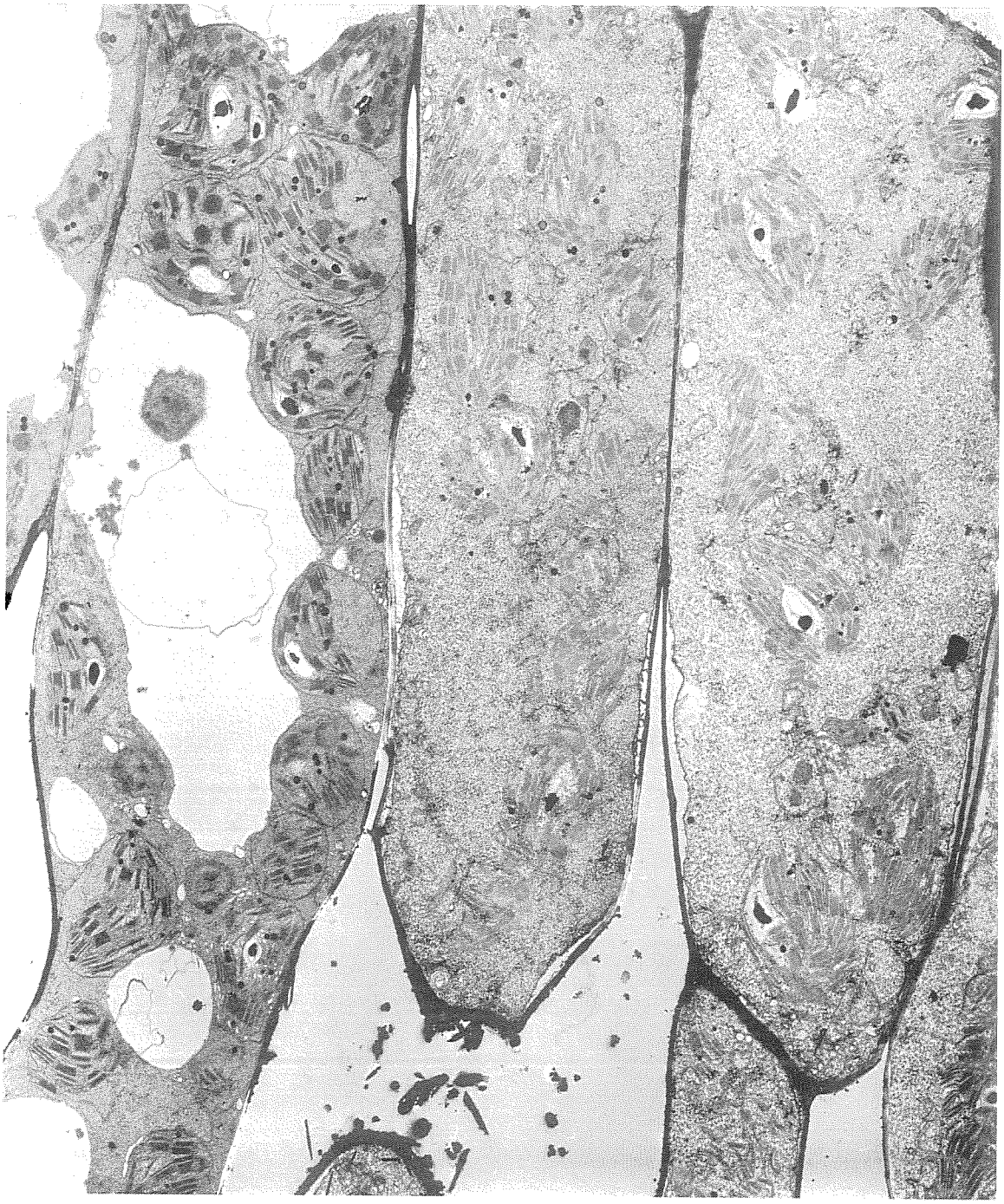

Plate IV

Cytopathic changes in the palisade parenchymatous cells of a tomato plant infected by PSTV. Note disintegration of chloroplast membrane systems, disappearance of tonoplasts and plasmolysis (arrow). ×6,400