ELECTRON MICROSCOPIC OBSERVATION OF HOST CELLS INFECTED WITH CUCUMBER MOSAIC VIRUS

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By

Tadao MISAWA and Yoshio EHARA

Department of Agronomy, Faculty of Agriculture*
Tohoku University, Sendai, Japan.

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Introduction

Bawden[1] and Johnson[4], in the studies of the unpurified extracted cucumber mosaic virus (CMV), reported the presence of rod shaped particles which occurred rather sparsely in their electron micrographs. But DeBruyn et al.[2], using a virus believed to be CMV, found spherical particles in preparations from *Nicotiana tabacum*. And then Sill et al.[8] found that the majority of preparatons from cucumber plants contained aggregates of characteristic ovoid to spherical particles having an approximate diameter of 35 mμ. But there appeared to be some question as to the identity of the virus used in their studies, because the method of purification of CMV had not been accurately established. Subsequently, Tomlinson et al.[7] reported a method of CMV purifying which involves homogenizing of infected tissue in a strong buffer containing a reducing agent and following centrifugating. They obtained spherical particles 40 mμ in diameter. More recently, Scott[9] found a purifying method of CMV that is superior in both purity and quantity, and yet the infectivity is held stable. He obtained polyhedral particles of 28–30mμ in diameter.

However, photographs of cucumber mosaic virus particles in the infected leaf cell have never been shown. We supposed that the CMV particles in the ultrathin sections of the infected tissue needed to be observed using a better technique. Thus we proceeded to the following experiments. This paper reports the morphology of CMV particles and the structural changes of chloroplast within the diseased cells.

Materials and Methods

1) Plant and Virus

The plant and the virus used were tobacco (var. Bright Yellow) and the

*Laboratory of Phytopathology

ordinary strain of cucumber mosaic virus. Healthy tobacco plants were grown in a Growth Cabinet (day temperature 29°C, moisture 80 percent, night temperature 24°C, night moisture 90 percent, illumination 12,000 lux and day time 12 hours). Upper surface of the leaves were inoculated mechanically with the juice of tabacco leaves infected by CMV. After the inoculation, the inoculated plants were transferred to the other Growth Cabinet (day temperature 25°C, moisture 80 percent, night temperature 20°C, moisture 25 percent, illumination 12,000 lux and day time 12 hours). Six, 8, 12 and 18 days after the inoculation, infected leaves were used for observations. Leaf tissues from healthy plants of the corresponding age and leaf position were examined as a control.

2) Electron microscopy

Ultrathin section was made by the method as Table 1. Small pieces of leaves (0.5 mm×0.5 mm) were cut and fixed for 4 hours in 2 percent OsO_4 buffered to pH 7.2 with veronal acetate (Palade[6]), and dehydrated by passage through a graded series of ethyl alcohol solutions in which the alcohol concentration was increased 10 percent every 2 hours. After the tissues were exposed to absolute alcohol for 3 hours, they were passaged in propylene oxide for 10 minutes, and then kept for 12 hours in a 1: 1 mixture of propylene oxide and Epon, according to the method of Luft[5]. After embedding in Epon, polymerization was carried out for 24 hours at 35°C, followed by 12 hours at 45°C, and finally by 24 hours at 60°C in No. 00 gelatin capsules. Sections about 200–300Å thick were cut with a Hitachi Ultra Microtome HUM-3 type and a Porter-Blum Ultra Microtome MT-1 type, using a glass knife. Ribbons of serial sections were retrieved from the collecting bath with collodion

Table 1. Method of Embedding by Epon*

1. Fixed with 1% OsO_4 veronal buffer solution.
2. Non washing, dehydration.
   - 30% ethyl alcohol, 10 minutes
   - 50 〃 〃 , 30 〃
   - 60 〃 〃 , 〃 〃
   - 70 〃 〃 , 〃 〃
   - 80 〃 〃 , 〃 〃
   - 90 〃 〃 , 〃 〃
   - 95 〃 〃 , 〃 〃
   - Absolute 〃 , 〃 〃 2 times
3. Clearing
   - Propylene oxide, 15 minutes, 2 times
   - Propylene oxide 3: Epon mixture 1, 1 hour
   - Propylene oxide 1: Epon mixture 1, 12 hours
4. Embedding and Polymerization
   - 35°C, 24 hours
   - 45°C, 8~12 hours
   - 60°C, 24 hours

* Epon Mixture (Equivalence of A and B)
A. Epon 812; 55cc DDSA; 100cc
B. Epon 812; 100cc MNA; 89cc
in addation of 2% DNP 30.

membranes, and mounted on copper specimen grids for the electron microscope, as described by Williams and Kallman[11]. The sections on the grids were stained with a saturated aqueous solution of uranyl acetate (Watson[10]) for 20 minutes. Some of the sections were mounted on copper grids with supporting film. After the grids were drained, specimen were examined with the Hitachi electron microscope HW-1 type and the Japan electron optics electron microscope JEM-7 type.

Results and Discussion

CMV particles within the host cell

Isodiametris particles of 20–25mμ and 10–15mμ in diameter were found in infected tobacco leaf cells. Ten to 15 mμ particles can be found also in healthy cells (Fig. 2), and its shape, size and distribution are similar to ribosomes. So these particles are assumed to be ribosome particles. Particles of 20–25 mμ in diameter were found in the infected cells (Fig. 3), but none were found in corresponding healthy cells. These particles have the same shape and simillar diminisions as particles known to be the purified CMV (Scott,[9]). Therefore, these particles are supposed to be the cucumber mosaic virus. These CMV particles exists in cytoplasm without a membrane bounded structure, and the arrangement of particles was either random or packed partially in crystalline arrays (Fig. 4). In the part of cytoplasm where these particles were mixed with ribosome, it is difficult to distinguish between virus particles and ribosomes, as the size between both particles, was slightly simillar. Furthermore, so far as the present study is concerned, the spherical particles believed to be CMV particles cannot be detected within chloroplast, nuclei and mitochondria, but are found only in the cytoplasmic matrix. These particles are approximatly spherical, but might be polygonal (Fig. 5). The fine structures of the particles are not very clear in any of the micrographs.

Appearence of CMV particles in the infected cell was greatly influenced by the fixing materials or the polymers in embedding. In the tissue embedded in methacrylate and its copolymer, the preservation of cytoplasmic matrix was very bad in comparison with Epon. And we could not find CMV particles in the infected tissus which had been embedded in such a polymer. Therefore it seems that methacrylate and its copolymer is easily dissoluted and sublimated by electron rays. In the case of fixation with $KMnO_4$, CMV particles could not be found in the tissue. Also $KMnO_4$ could not fix ribosome.

These observations were carried out by Doi et al.[3] conccurently with us and the same result was obtained.

Abnormality of chloroplast

With the lapse of time after infection, the chloroplast of cells in the diseased tissue showed marked distortion and degeneration as compared with the chloroplasts

(Fig. 1) from healthy cells. Six to 8 days after inoculation, CMV particles had existed already in cytoplasm. And osmiophilic bodies in the diseased chloroplast became much larger, although no differences were apparent between extramembrane, grana lamellae and stroma lamellae in chloroplast of diseased and healthy cells (Fig. 6. 7). Twelve days after inoculation, these chloroplasts in diseased cells were highly disorganized, namely, clumping of stromatic material and abnormal structure, and vacuolated parts increases. Moreover, the expanded osmiophilic bodies faded away (Fig. 8–10). Eighteen days after inoculation, the chloroplast lamellae collapses into particles, the stromatic material is compeletely destroyed, and the particles from the destruction accumulate near the membrane. All osmiophilic bodies disappear, but the grana lamellae exists scanty (Fig. 11. 12).

All chloroplasts in infected cell are not disintegrated simultaneously. Integrated and disintegrated chloroplasts exist together in the same cell (Fig. 13). With the lapse of time after infection, the number of disintegrated chloroplasts increase and the normal chloroplasts per cell decrease. In the diseased cell, the disintegrated stage of the chloroplast accords with the decreasing stage of chlorophyll contents of the tissue (unpublished). Therefore, it was concluded that the decrease of chlorophyll contents by infection was caused by the structural disintegration of chloroplasts.

Summary

Ultrathin sections of leaf infected by CMV were examined with electron microscope. Spherical particles, about 20–25mμ in diameter, were found in the cytoplasmic matrix. But in cells of none inoculated control plants, such particles were absent. Also the particles had the same shape and similar dimentions as particles known to be the purificated CMV. Therefore, these particles were supposed to be CMV particles. These CMV particles exist in cytoplasm without a membrane bounded structure, and the arrangement of particles was either random or packed partially in crystalline array. The spherical particles believed to be CMV particles could not be detected the chloroplast, nuclei and mitochondria.

With the lapse of time after infection, the chloroplast of diseased cells showed marked distortion and degeneration as follows. Six to 8 days after inoculation, osmiophilic bodies in the chloroplast expanded. Twelve days after inoculation, chloroplast appeared highly disorganized with dissolution of stromatic material and vacuolated portion increase. Also the expanded osmiophilic bodies faded away. Eighteen days after inoculation, chloroplast membrane and stromatic lamellae collapse into particles, and grana lamellae exists scanty. Such disintegrated chloroplasts exist together with integrated chloroplasts in the same cell.

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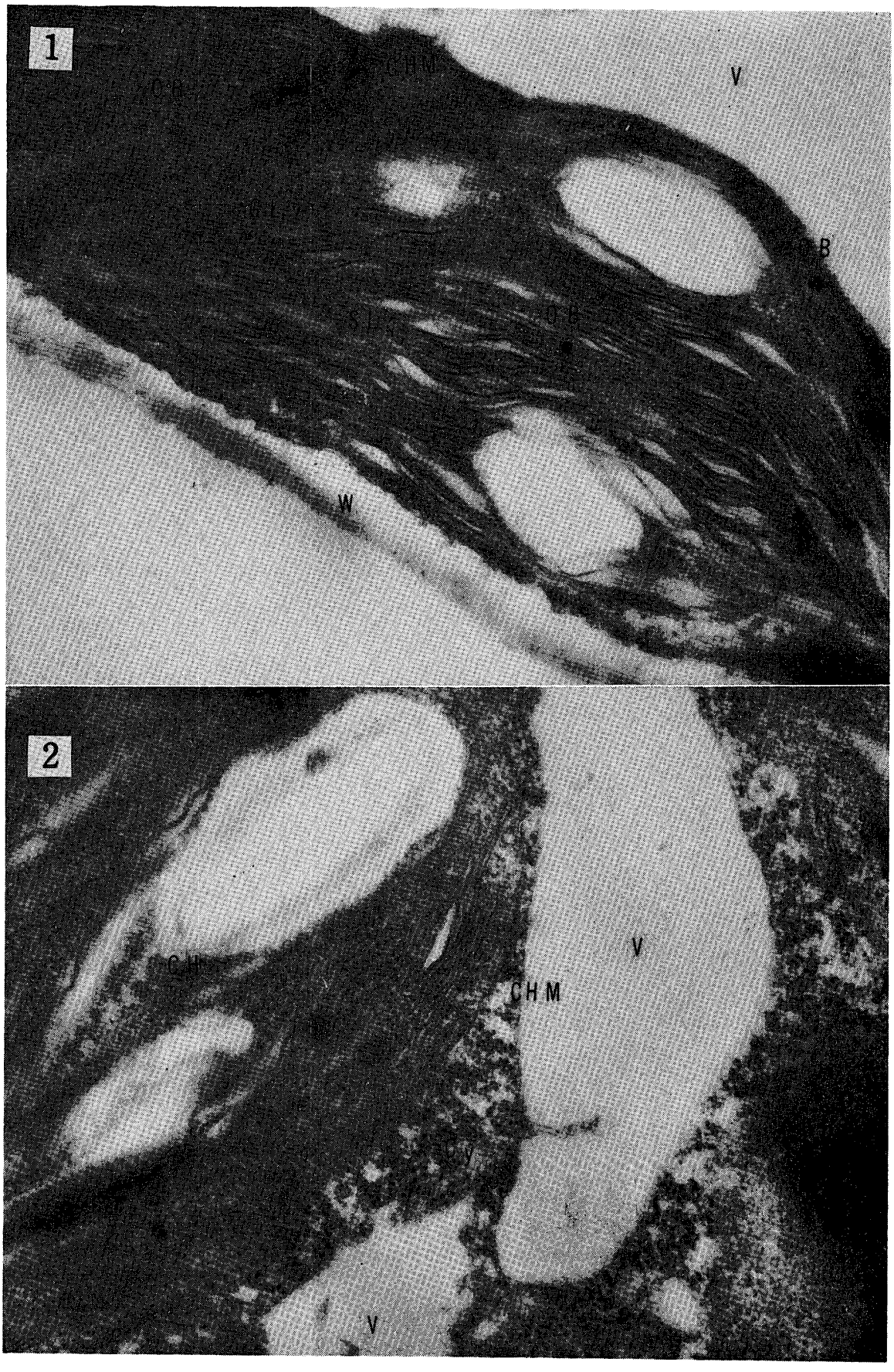
Plate 1

Fig. 1 and 2. Healthy chloroplast (Fig. 1) showing typical organelle arrangment. × 24,000. Healthy cytoplasm (Fig. 2) having ribosome particles in diameter of about 10–15 mμ. ×58,000. CH: Chloroplast, CHM: Chlroplast membrane, OB: Osmiophilic bodies, GL: Grana lamellae, SL: Stroma lamellae, W: cell wall, V: Vacuole, Cy: Cytoplasm.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V
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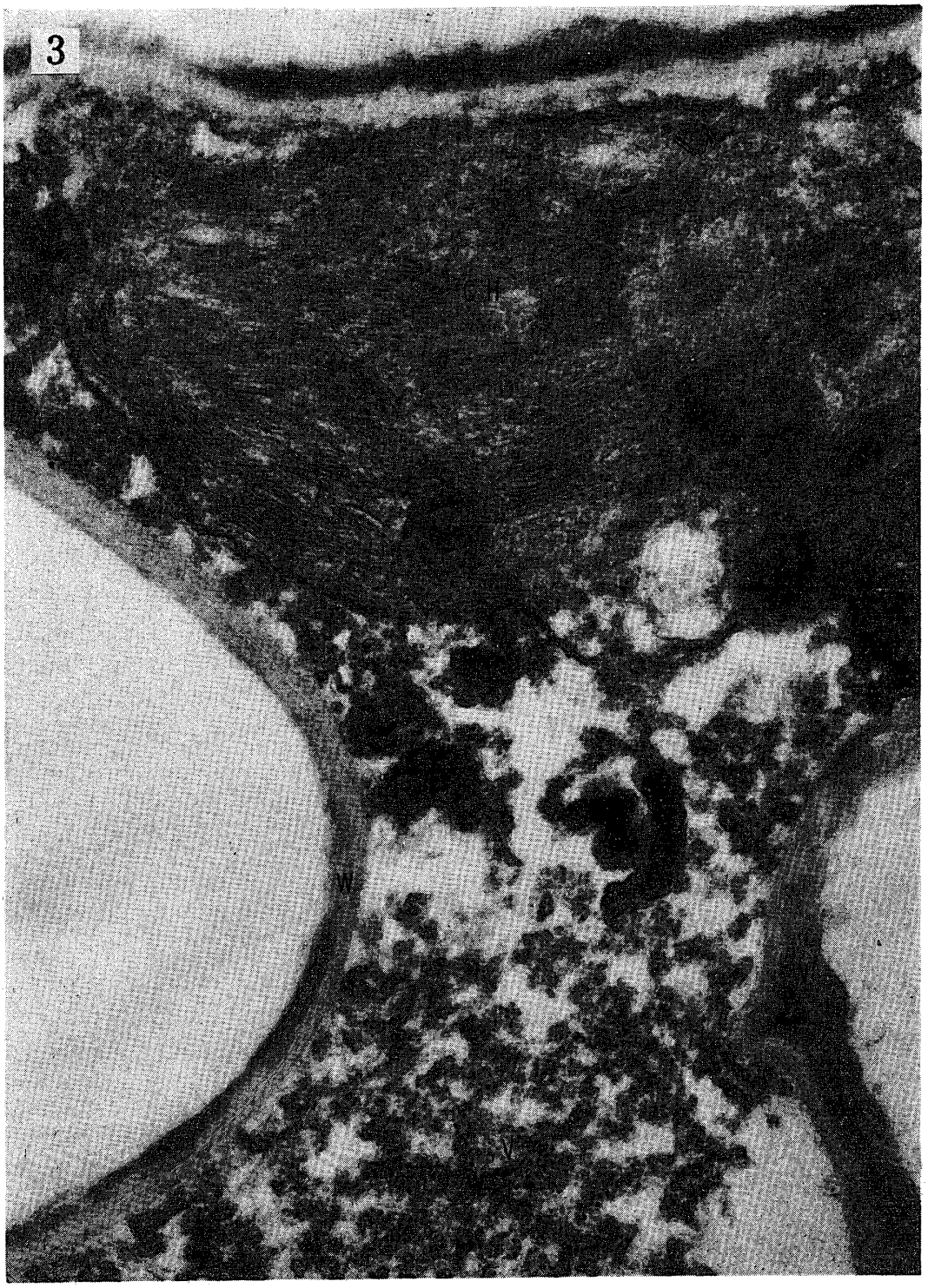
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Plate 2

Fig. 3. Electron micrograph of a section of CMV infected tobacco leaf cell. CMV particles (V) were arranged either at random or arranged partially in a crystalline array within cytoplasmic matrix. W: Cell wall, CH: Chloroplast. × 36,000.

Plate 3

Fig. 4. Electron micrograph showing the arrangment of CMV particles. × 54,000

Fig. 5. Electron micrograph showing morphology of individual particles. These particles seem to be hexagonal (arrow). × 100,000.


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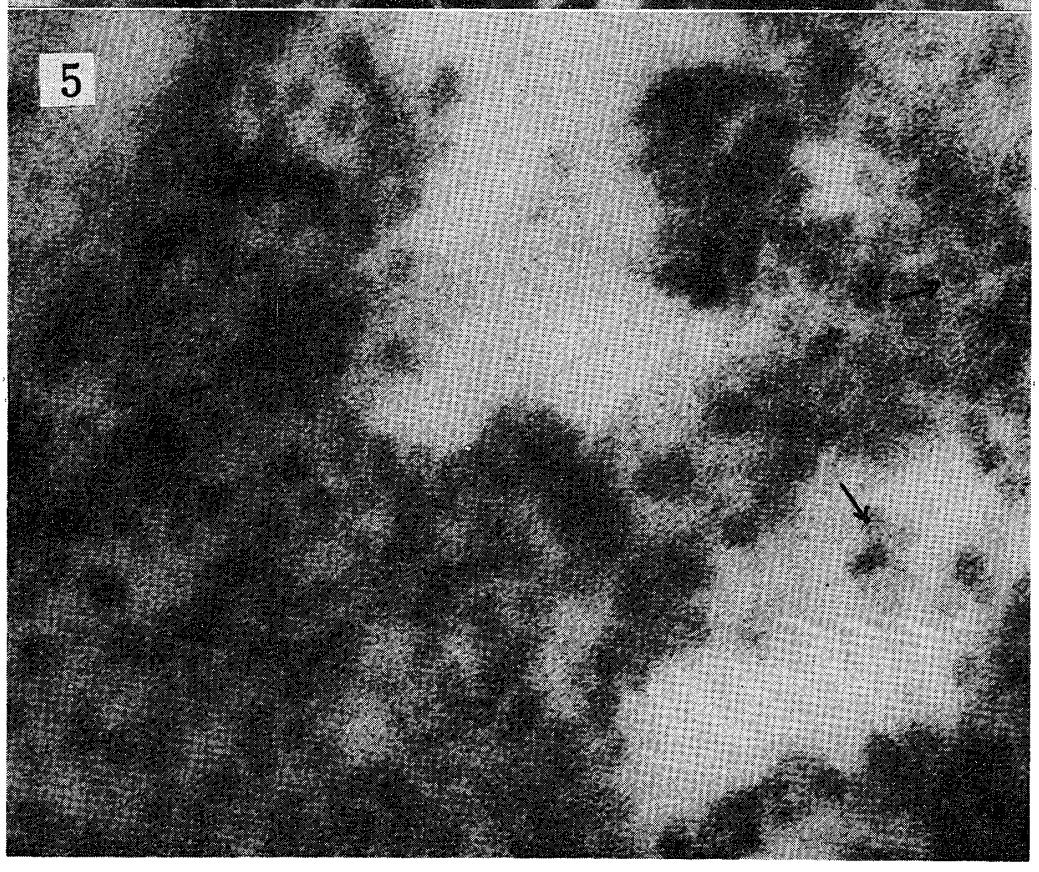
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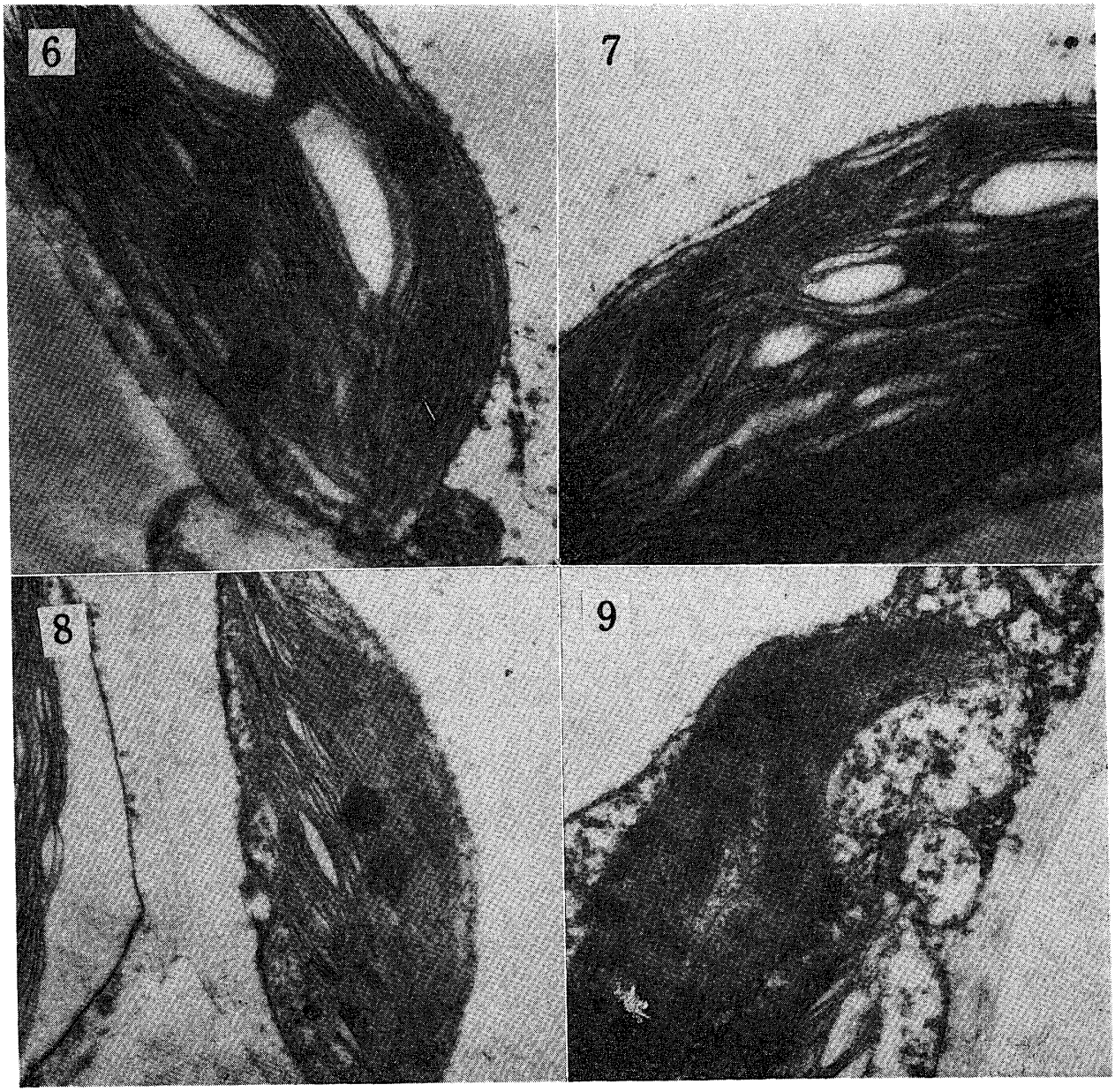
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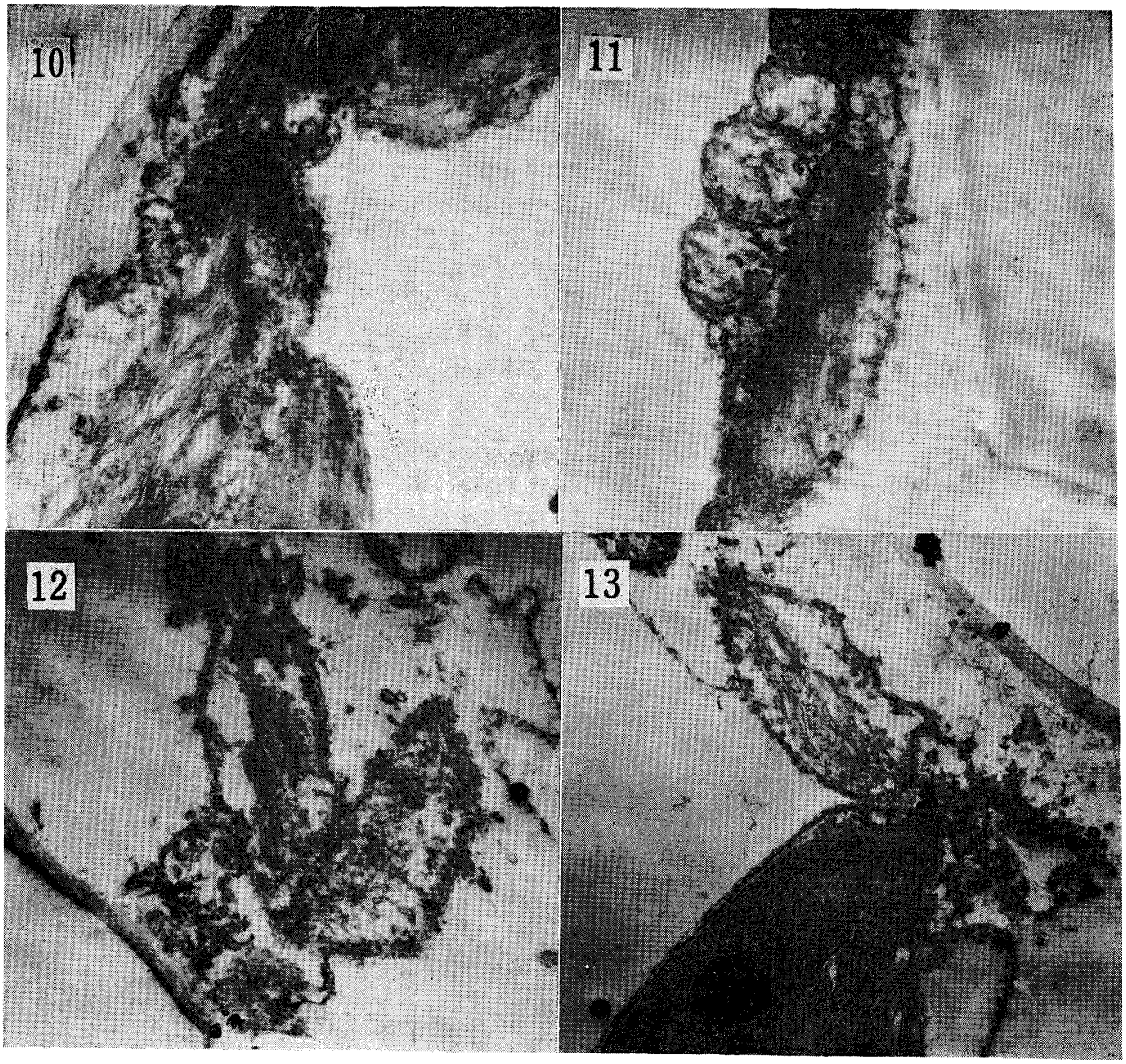
Plate 4

Fig. 6–13. Chloroplast from diseased plant. Expanding of osmiophilic bodies (Fig. 6–7). Collapsing of chloroplast membrane (Fig. 8). Clumping and dissolution of stromatic material, and vacuolation (Fig. 9–10).

Plate 5

Chloroplast collapses to particles, and stromatic material destructs and such destroyed particles accumulate near the collapsed membrane (Fig. 11–12). Coexistence of the integrated chloroplast and disintegrated one (Fig. 13). × 12,000.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