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Ultrastructure of *Cymbidium* Leaf Tissue Systemically Infected with Odontoglossum Ringspot Virus

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오돈토글로썸 윤문 바이러스에 감염된 *Cymbidium* 잎조직의 미세구조

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적 요

오돈토글로썸 윤문 바이러스(ORSV)에 감염된 한국 자생춘란(*Cymbidium goeringii*) 초박절편의 전자현미경 관찰에서 ORSV 입자들은 집적되거나 환형상 평면구조로 다양한 크기의 불규칙적인 응집된 다발상들이 관찰되었다. 바이러스 입자들은 원형질체에서 다수 존재하였고, 세포벽과 원형질막 사이에서도 존재하였으며, 이들은 주로 응집된 평행상과 불규칙적인 산재된 형태로 관찰되었다. X-체 및 paramural body는 원형질막 주변에 존재하였고 액포와 유사한 낭상 구조체에 싸여있었다. 바이러스에 감염된 조직의 세포벽은 확장되어 있었다. ORSV에 감염된 세포내 엽록체는 불규칙적인 모습을 보였다. 미토콘드리아, 핵, 액포, 소포 및 기타 세포내 소기관들내에서는 바이러스 입자를 볼 수 없었다. 원형질연락사(plasmodesmata)는 다소 확장되고, 바이러스 입자들이 이들 원형질연락사 주변에 존재하였다.

INTRODUCTION

The Orchidaceae is generally considered the largest family of ornamental plants with about 30,000 species representing a highly diverse group (Helmut *et al.*, 1992). Most commercially cultivated orchid genera in Korea were *Cymbidium, Cattleya, Dendrobium, Oncidium* and *Phalaenopsis*. In 1993, Korea imported approximately 9.9 millions sprays of *Cymbidium* spp. from China, Japan and Taiwan, and 1.5 millions sprays and blossoms of *Dendrobium phalaenopsis* from Thailand. Virus diseases are found frequently on orchids. Viral infections are mostly caused by cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV) (Francki, 1970; Park *et al.*, 1990a; Paul, 1975).

ORSV is a member of the tobamovirus group of plant viruses and is known as an economically important pathogen of the Orchidaceae (Paul, 1975; Zettler *et al.*, 1990). Its host range is restricted in the Orchidaceae. Tobamoviruses have 300×18nm rigid

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-rod particles, and contain a 6.4kb positive-sense single-stranded genomic RNA that is capped at its 5' end and has tRNA-like structure at its 3' end. ORSV was first isolated from *Rossioglossum grande,* formerly called *Odontoglossum grande,* by Jensen and Gold (1951). The virus is transmitted mechanically.

Studies on biological, serological, physico-chemical properties, and ultrastructural investigations of infected tissues of ORSV have been reported (Chang *et al.*, 1991; Christie and Edwardson, 1977; Jensen and Gold, 1951; Milicic and Stefanac, 1971; Park *et al.*, 1990b, c; Paul, 1975; Wisler *et al.*, 1982). The morphology and staining reactions of ORSV-induced crystalline, paracrystalline, and angled-layer aggregate inclusions are diagnostic for infection by this virus in comparisons with inclusions induced in orchids by viruses in other groups (Edwardson and Zettler, 1986). The induction of cytoplasmic crystalline inclusions consisting of virions aligned parallel to each other and to the lateral faces of the inclusion is a main characteristic of the tobamoviruses (Christie and Edwardson, 1977; Matthews, 1991; Toussaint *et al.*, 1984).

The purposes of the present researches were identification of ORSV and investigation of the ultrastructures of *Cymbidium* leaf tissue infected by ORSV.

MATERIALS AND METHODS

1. Source of plant

The tissue sample was leaves of 2-years-old *Cymbidium goeringii* Reichenbach from a commercial orchid farm. It is cool-growing native Oriental orchid which is popular in Korea. The leaves of the orchid showed necrotic spots, mild mosaic and stripes. The presence of ORSV in collected plant was checked by serological assays and electron microscopic observation (Park *et al.*, 1990b, c). The plant was maintained in the greenhouse.

2. Ultrathin section

The leaf tissues of ORSV infected *Cymbidium goeringii* were fixed in 5% glutaraldehyde in 0.1M phosphate buffer, pH 6.8 and cut into 1mm^2 pieces with sterilized razor blade. The disks were transferred to fresh fixing solution (2.5% paraformaldehyde -glutaraldehyde in the phosphate buffer) for 30min at room temperature under vacuum, and kept at 4℃ for 3 hrs. The samples were washed with the phosphate buffer for 5min, three times and post-fixed in 1% (w/v) osmium tetroxide in the phosphate buffer for 2 hrs. After three times washes in the same buffer, they were dehydrated in ethanol and propylene oxide series, and were embedded in Epon 812-Araldite mixture (Anderson and Andre, 1968) polymerizing at 60℃ for 72 hrs. The samples were cut into ultrathin sections with a diamond knife on an LKB V-type ultramicrotome. The thin sections were mounted on carbon Formvar coated grids. They were stained with 2% aqueous uranyl acetate for 15min and counterstained with 0.2% lead citrate for 5min. These stained specimens were examined and photographed with JEM 100 CX-II electron microscope operated at 80KV.

RESULTS

1. Particle morphology

The morphology of the virus particles was rigid rod-shaped of tobamoviruses. The central canal was observed in the virus particle. The average length of the virus particle was 300nm, and width was 18nm (Fig. 1).

2. Ultrastructures

A numbers of ORSV particles were observed in the sections of the leaf tissues. Some cells filled with large aggregates of virus particles (Figs. 2, 3). Intracellular ORSV particles appeared as bundles of irregular aggregates of various length which were

Fig. 1. Odontoglossum ringspot virus (ORSV) particles by immunosorbent electron microscopy in *Cymbidium goeringii*. Scale bar represents 100nm.

stacked plates and rounded plates (Figs. 2, 5). Virus particles were also found in the cytoplasm in electron clear zones and they were distributed between cell wall and plasma membrane (Figs. 4, 5). The virus-associated X-bodies and paramural bodies were observed near the cell membrane and these contained vacuole-like cavities (Figs. 6, 7). The viruses mainly clustered in parallel aggregates and accumulated in the cytoplasm and often formed crystalline arrays (Figs. 2, 3, 5, 7). The thickening of cell wall in infected tissue was observed, and the plasma membrane arrays were built up relatively loosely (Fig. 6). The damaged chloroplast of ORSV infected cell was irregular (Figs. 6, 7), but some of the chloroplasts were apparently healthy. Virus-like particles were found, though less frequently, in the damaged chloroplast (Figs. 6, 7). Vesiculation and distortion of the mitochondria were seen in the virus-infected cell (Fig. 3). The membrane of the mitochondrion was partially swollen (Figs. 3, 6). No virus particle was seen in the mitochondria, nuclei, vacuoles, vesicles or other organelles. The plasmodesmata were slightly enlarged, and virus-associated granules of paramural bodies were present around it (Fig. 6).

DISCUSSION

The members of the tobamovirus group are very stable particles that usually occur in high concentrations in their host tissues. They are mechanically transmissible, and are naturally transmitted by contact through wounds. Unlike other plant virus group, there might be no efficient natural vectors.

ORSV is one of the several tobamoviruses found in orchids (Paul, 1975). The electron microscope provides valuable information about inclusions and their environment at the ultrastructures (Christie and Edwardson, 1977; Edwardson and Christie, 1978; Martelli and Russo, 1977). In many cases inclusions have such a distinctive appearance that they may be used to identify a specific virus. Tobamoviruses induce several types of inclusions (Christie and Edwardson, 1977; Toussaint *et al.*, 1984). These unique inclusions consist of aggregates of virus particles that have been described as hexagonal, stacked, rounded plates, paracrystallines, and angled-layer aggregates. In addition to the virus aggregates certain tobamoviruses induce inclusions known as X-bodies.

ORSV particles were flexible because preparations contained short-length as well as normal-length. ORSV concentration in leaf tissue was rich and virus particles were found in most parts of the cytoplasm of the diseased plant. As with other tobamoviruses, intracellular ORSV particles appeared as bundles of irregular aggregates of variable length which were stacked plates or rounded plate. The X-bodies were also observed near the cell membrane. Association of ORSV with leaf cells, especially with chloroplast, may be responsible for the virus symptom expression. ORSV was reported to induce rounded plates (Milicic and Stefanac, 1971). Christie and Edwardson (1977) reported that an orchid strain of TMV (TMV-O) also induced stacked, rounded plates, and large numbers of para-

crystals in the old tissues infected with an TMV-O. Granett and Shalla (1970) reported that X-bodies differed quantitatively in three strains of TMV.

The purposes of most workers studying the cytopathology of tobamovirus-infected cells have been to obtain evidence on the sites of synthesis and assembly of the virus particles (Granett and Shalla, 1970; Martelli and Russo, 1977; Milicic and Stefanac, 1971). The evidence is still ambiguous, though it is clear that the particles accumulate in the cytoplasm and that the other organelles are not altered significantly except as a secondary result of infection. A number of cytological studies of plant virus infections have reported failure to detect inclusions. This may be the result of inadequate sampling. Inclusions can easily be missed in samples that are taken at the wrong stage of development, since at early stages of virus infection they may not have formed and at later stages they might disappear.

Many virus infected orchids showed no foliage and flower symptoms. Virus-free plants as source for tissue culture is very important. For virus-free mother plant, bioassays, serological and electron microscopy tests should be conducted.

ABSTRACT

In ultrathin section of the tissue of odontoglossum ringspot virus (ORSV)-infected *Cymbidium goeringii* Reichenbach, ORSV particles appeared as bundles of irregular aggregates of various length which were called stacked plates or rounded plates. Virus particles were found in the cytoplasm in electron clear zones and they also found between cell wall and plasma membrane. They mainly clustered in parallel aggregates and sometimes oriented randomly. The X-bodies and paramural bodies were observed near the cell membrane and these contained vacuole-like cavities. The cell wall of infected tissue expanded largely. Some chloroplast in ORSV infected cell was irregular. No virus particle was present in mitochondria, nuclei, vacuoles, vesicles or other organelles. The plasmodesmata slightly enlarged, and virus-associated granules were present around it.

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REFERENCES

Anderson, W.A. and Andre, J., 1968. The extraction of some cell components with pronase and pepsin from thin sections of tissue embedded in an Epon -Araldite mixture. J. Microscopie 7, 343-354.

Chang, M.U., Chun, H.H., Baek, D.H. and Chung, J. D., 1991. Studies on the viruses in orchids in Korea. 2. Dendrobium mosaic virus, odontoglossum ringspot virus, orchid fleck virus and unidentified potyvirus. Korean J. Plant Pathol. 7, 118-129.

Christie, R.G. and Edwardson, J.R., 1977. Light and electron microscopy of plant virus inclusions. Florida Agricultural Experiment Stations Monograph Series No. 9. 150pp. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA.

Edwardson, J.R. and Christie, R.G., 1978. Use of virus induced inclusions in classification and diagnosis. Ann. Rev. Phytopathol. 16, 31-55.

Edwardson, J.R. and Zettler, F.W., 1986. Odontoglossum Ringspot Virus. 233-247pp. In: The Plant Viruses Vol. 2. The Rodshaped Plant Viruses. edited by M.H.V. Van Regenmortel. Plenum Press.

Francki, R.I.B., 1970. Cymbidium mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 27, Kew,

England.

Granett, A.L. and Shalla, T.A., 1970. Discrepancies in the intracellular behavior of three strains of tobacco mosaic virus, two of which are serologically indistinguishable. Phytopathology 60, 419-425.

Helmut, B., Cribb, P. and Launert, E., 1992. The Manual of Cultivated Orchids Species. 3rd Ed. American Orchid Society Book Department, Florida. 585pp.

Jensen, D.D. and Gold, H., 1951. A virus ring spot of *Odontoglossum* orchid: symptoms, transmission, and electron microscopy. Phytopathology 41, 648-653.

Lesemann, D.-E., Bozarth, R.F. and Koenig, R., 1980. The trapping of tymovirus particles on electron microscope grids by adsorption and serological binding. J. Gen. Virol. 48, 257-264.

Martelli, G.P. and Russo, M., 1977. Plant virus inclusion bodies. Adv. Virus Res. 21, 175-266.

Matthews, R.E.W., 1991. Plant Virology, 3rd ed. 835pp. Academic Press.

Milicic, D. and Stefanac, Z., 1971. Cell inclusions of the cucumber green mottle mosaic virus and the odontoglossum ringspot virus. Acta Botanicum Croatica 30, 33-40pp.

Park, W.M., Kim, W.G., Ryu, K.H., Yoon, K.E., Kwack, B.H. and So, I.S., 1990a. Detection of odontoglossum ringspot virus and cymbidium mosaic virus from cultivated orchids by immunosorbent electron microscopy. J. Kor. Soc. Hort. Sci. 31, 417-422.

Park, W.M., Yoon, K.E., Chung, S.Y. and Ryu, K.H., 1990b. Identification of odontoglossum ringspot virus isolated from *Cymbidium goeringii* Reichenbach in Korea. Korean J. Plant Pathol. 6, 387-392.

Park, W.M., Yoon, K.E., Chung, S.Y. and Ryu, K.H., 1990c. Purification and serological detection of odontoglossum ringspot virus isolated from *Cymbidium goeringii* in Korea. Korean J. Plant Pathol. 6, 474-481.

Paul, H.L., 1975. Odontoglossum ringspot virus. CMI/AAB Descriptions of Plant Viruses No. 155, Kew, England.

Toussaint, A., Dekegel, D. and Vanheule, G., 1984. Distribution of odontoglossum ringspot virus in apical meristems of infected Cymbidium cultivars. Physiol. Plant Pathol. 25, 297-305.

Wisler, G.C., Zettler, F.W. and Purcifull, D.E., 1982. A serodiagnostic technique for detecting cymbidium mosaic and odontoglossum ringspot viruses. Phytopathology 72, 835-837.

Zettler, F.W., Ko, N.J., Wisler, G.C., Elliott, M.S. and Wong, S.M., 1990. Viruses of orchids and their control. Plant Dis. 74, 621-626.

ABBREVIATION

Ch : Chloroplast
CW : Cell wall
M : Mitochondrion
N : Nucleus
P : Paramural body
PD : Plasmodesmata
PM : Plasma membrane
T : Tonoplast
V : Vacuole

FIGURE LEGENDS

Fig. 2. Ultrastructure of ORSV-infected parenchyma cell of *Cymbidium goeringii*, showing masses of virus particles in the cytoplasm. The cell on the middle contains stacked and rounded plates (arrow). Scale bar represents 1μm.

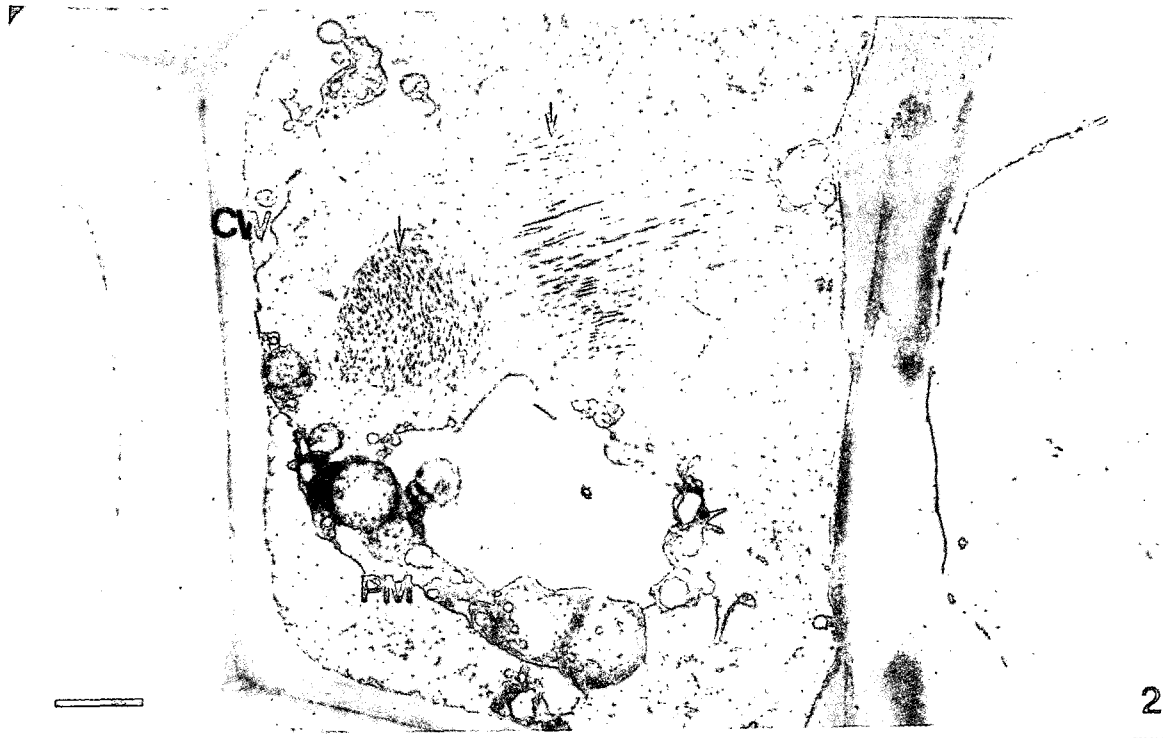
Fig. 3. *Cymbidium goeringii* leaf cell infected with ORSV, showing randomly scattered virus particles (arrow) in the cytoplasm and vesiculation of the mitochondrion (M). Scale bar represents 1μm.

Fig. 4. ORSV-infected mesophyll cell of *Cymbidium goeringii,* showing masses of virus particles in the cytoplasm and between cell wall (CW) and plasma membrane (PM). Scale bar represents 1μm.

Fig. 5. Ultrastructure of ORSV-infected mesophyll cell of *Cymbidium goeringii,* showing masses of virus particles (arrow) in the distribution between cell wall (CW) and plasma membrane (PM). Scale bar represents 1μm.

Fig. 6. *Cymbidium goeringii* leaf cell infected with ORSV, showing vesiculated mitochondrion (M), characteristic stacking of virus particles (arrow), the thickenings of cell wall (CW) and plasmodesmata (PD). The X-bodies and paramural bodies (P) were observed near the cell membrane and these contained vacuole-like cavities. Scale bar represents 1μm.

Fig. 7. Thin section of a *Cymbidium goeringii* leaf cell infected with ORSV, showing characteristic stacking of virus particles (arrow). The damaged chloroplast (Ch) was irregular. Virus particles were found in the chloroplast. Scale bar represents 1μm.



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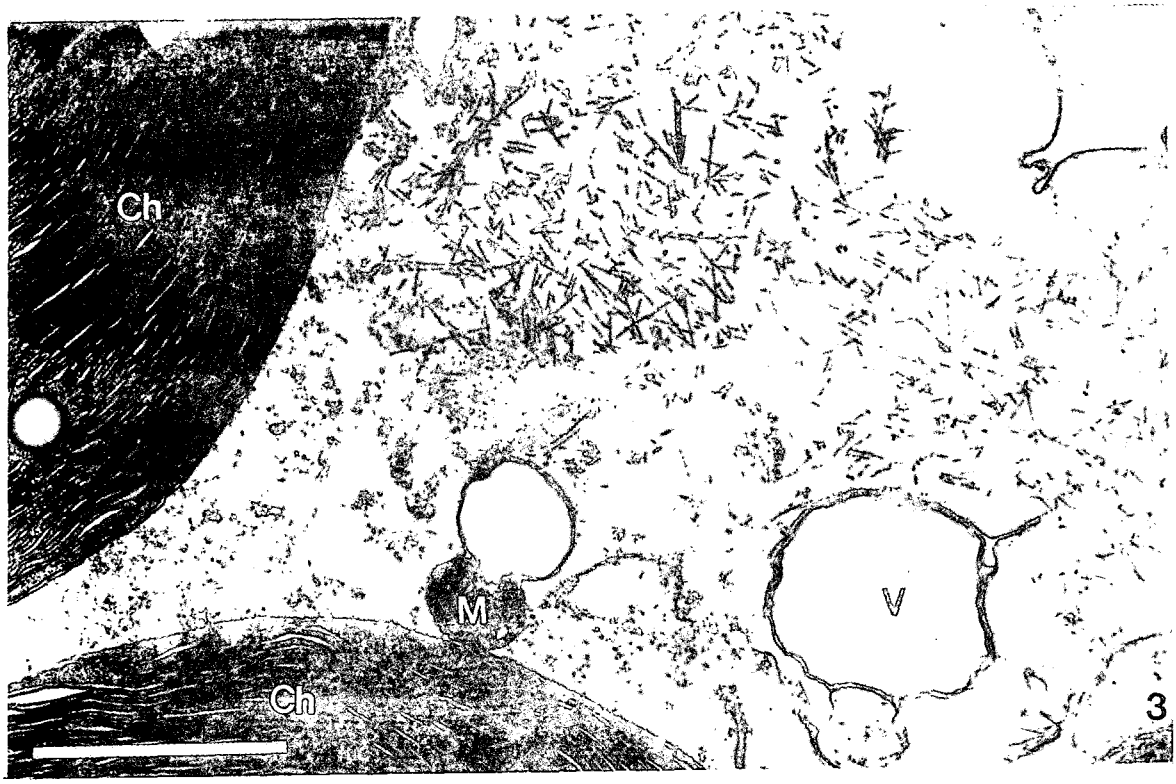

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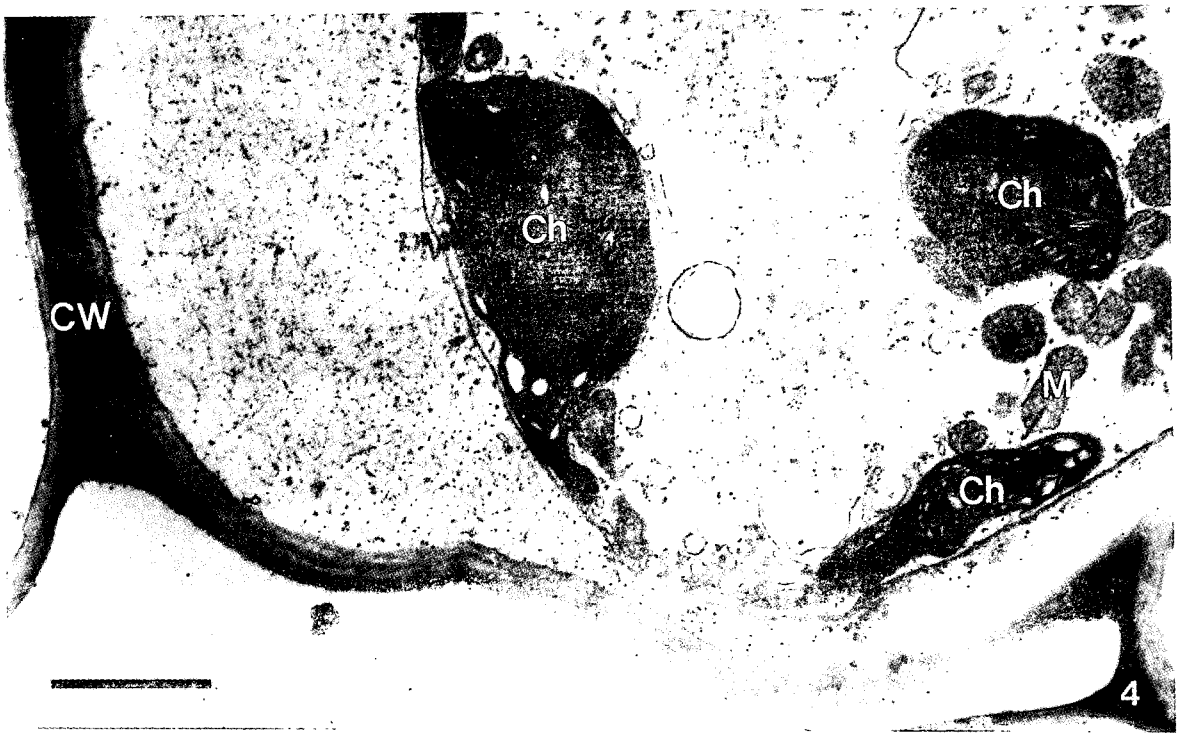
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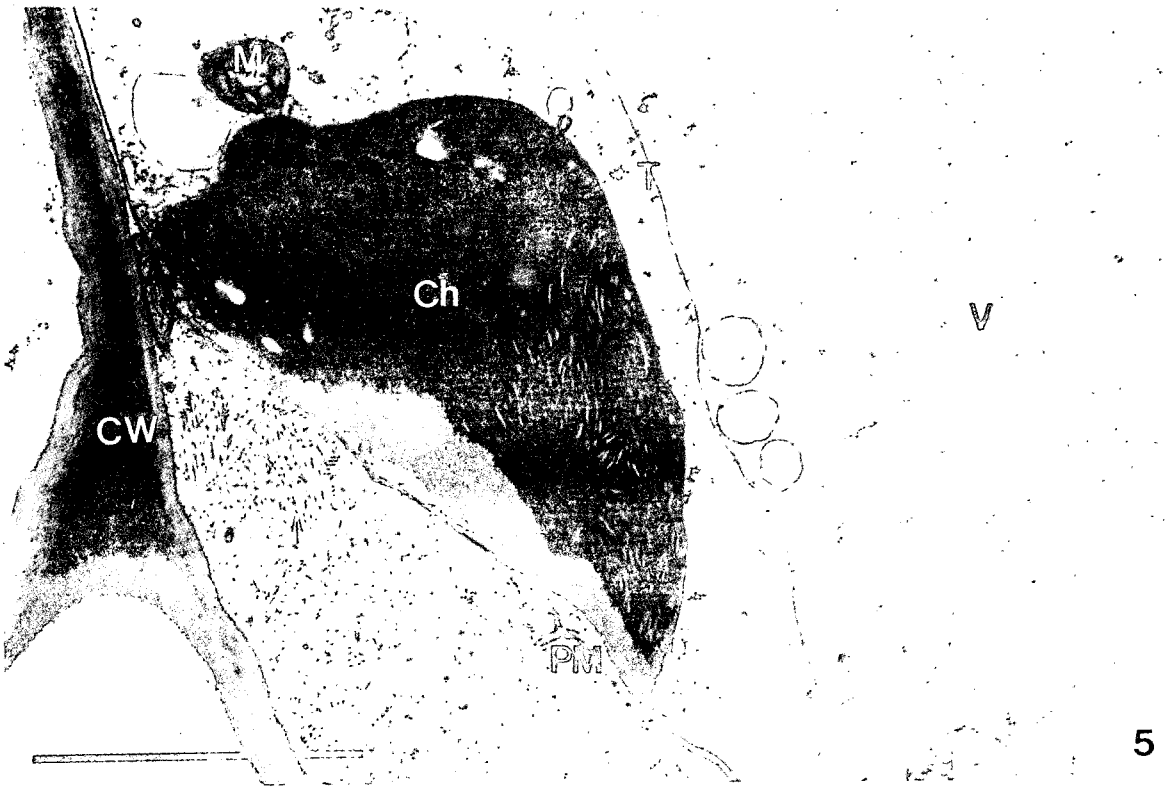
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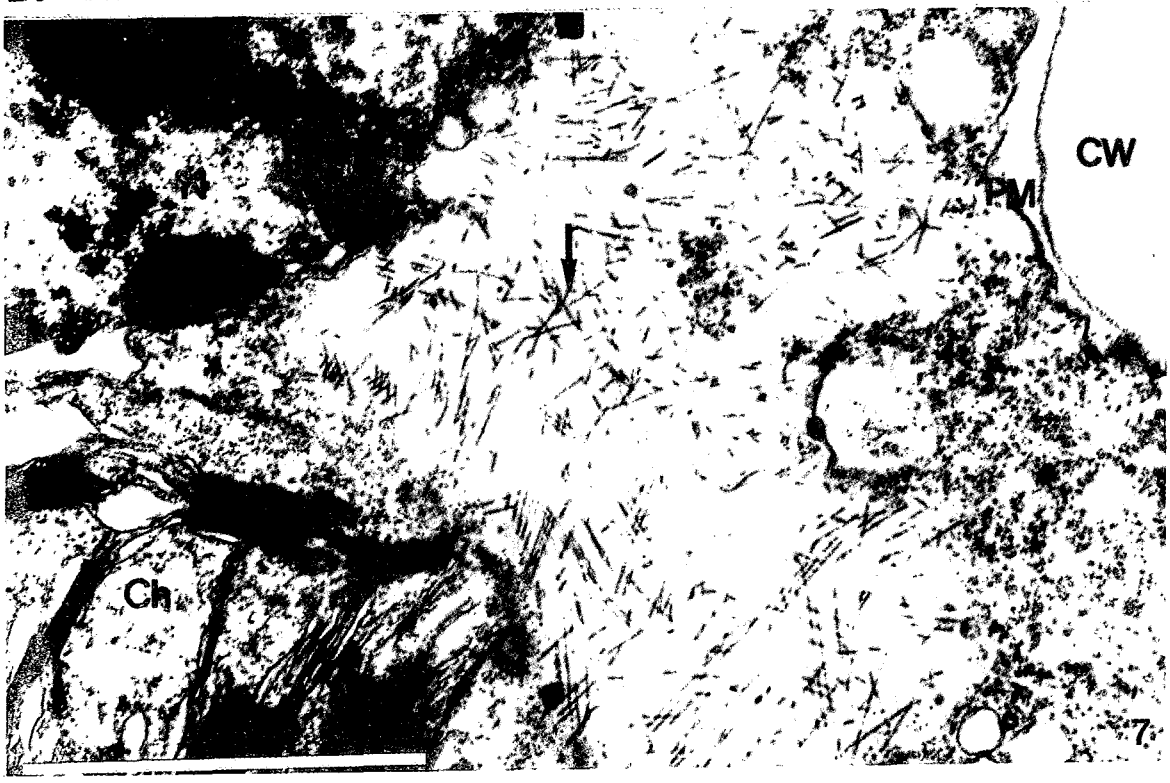
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