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Foliar ultrastructure of Korean *Orostachys* species.

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韓國産 바위솔屬 葉肉組織의 微細構造

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요 약

CAM대사를 수행하는 것으로 추정되는 한국산 바위솔속식물 3종(난장이바위솔, 바위솔, 등근바위솔)의 엽육조직이 전자현미경적으로 연구되었다. 다육질성의 이들 엽육조직은 주로 세포의 크기가 큰 수분저장 세포들로 구성되어 있고 세포간극이 잘 발달되어 있다. 세포내 또는 세포간극에는 점액성 분비물질들이 분포하였다. 수분저장 세포에는 하나 또는 여러 개의 큰 액포가 있고 이들이 세포체적의 대부분을 차지하여 세포질은 세포벽 주위에서 매우 적게 분산되어 나타났다. 세포질내에는 다수의 엽록체, 미소체, 미토콘드리아, 소포체, 골지체 등의 전형적인 세포소기관외에 엽록체주변에서 관찰되는 세포질의 특이한 분포양상을 비롯하여 세포벽과 분리되어 나타나는 파상(undulation or invagination)의 세포막과 이중막으로 둘러싸인 원형질막 기원소포(plasmalemmasome) 및 수초상구조(myelin-like structure), 액포상구조(vacuole-like structure) 또는 낭상구조(blad-der-like structure) 등이 발견되었다. 특히 파상의 세포막 및 액포막의 신장으로 형성된 액포상 또는 낭상구조와 엽록체주변 세포질의 특이한 분포양상 등은 주목할 만한 것으로 이들의 특성 및 구조는 CAM대사와 연계되어 자세히 연구되어야 할 것이다. 이와 같은 엽육조직의 구성 및 미세구조적 특성으로 보아 본 바위솔속 식물들은 CAM대사를 수행하는 식물군으로 사료된다.

Key words : Foliar ultrastructure, *Orostachys* species, CAM features

INTRODUCTION

The genus (family Crassulaceae) contains succulent plants adapted to a variety of dry environments. A number of species in this genus appear to exhibit Crassulacean acid metabolism (CAM), as do representatives from a few other genera in

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the family. Three species are known in Korea (Lee, 1980) and they have features which make it possible to survey the occurrence of the CAM mode based on morphology and the habitats in which they occur. The CAM mode is suspected to occur since the genus contains species that grow along the shore within the range of sea water spray (Kim *et al.*, 1995). A high NaCl concentration is known to affect the photosynthetic pathway in plants (Osmond, 1978; Kluge and Ting, 1978). According to Kluge and Ting (1978), most of the Crassulaceae, in all probability, have CAM or at least may perform CAM under certain environmental conditions. CAM plants are usually succulents that are adapted to survive under drought conditions.

The typical CAM photosynthetic cells are large and thin-walled structures that exhibit narrow peripheral cytoplasm adhering to the cell walls. These cells appear as empty spheres in the microscope because of the presence of huge water-storing vacuoles that occupy the most of the cell volumes. As a result, the number of chloroplasts and other organelles per cell appears small in comparison with that of non-succulent plants. However, this may be illusionary because of the large cell size (Kluge and Ting, 1978).

The ultrastructure of CAM plants has been less extensively investigated and most electron microscope studies of CAM have emphasized the ultrastructure of chloroplasts. Further, ultrastructural studies of leaves of CAM plants are not abundant. Those which have been done, mostly focused on features of the chloroplasts and microbodies (Kapil *et al.*, 1975; Lee and Thomson, 1973; Rivera and Arnott, 1982; Salema and Brandao, 1978; Santos and Salema, 1981; Thomson and Platt, 1973; Vaughn and Wilson, 1981). The present study was undertaken to examine the ultrastructure of leaf succulent species and to find any characteristics of CAM structures reported in other CAM plants. The present paper reports on various cellular structures found in leaves of three species grown under natural xeric conditions.

MATERIALS AND METHODS

Small pieces of tissue were dissected from the middle portion of healthy mature leaves of *Orostachys japonicus* A. Berger, *O. malacophyllus* Fisch. and *O. sikokianus* Owhi. The tissues were fixed in a mixture of 1% paraformaldehyde and 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 6.8~7.2) at room temperature. After fixat-ion for 3 hours followed by a short rinse in buffer, the material was postfixed with 2% OsO_4 in the same buffer for 2 hours to overnight at 4 °C. After postfixation followed by a 30 min rinse, specimens were dehydrated in a graded acetone series and embedded in Spurr's low viscosity resin (Spurr, 1969). Thin sections were cut on a RMC 7000 ultramicrotome with diam-ond knives. The sections were stained with 2% aqueous uranyl acetate for 45 min followed by a 30 min staining with lead citrate. Ultrathin sections were viewed in a Phillips EM201 microscope.

RESULTS

Ultrastructural observations were made on leaves of three Korean *Orostachys* species which occur in distinctly xeric habitats. These species, however, showed rather simple foliar structures. Relatively undifferentiated mesophyll cells, water-storing mesophyll tissue, occupied most of the internal leaf volume. Each water-storing mesophyll cell contained a large central vacuole

with a thin peripheral layer of cytoplasm. The ultrastructure of leaf blades of all three *Orostachys* species is similar and the description here applies to all three species unless otherwise specified. An intercellular space system was well developed in all species. In *O. sikokianus*, corners of intercellular spaces were frequently observed to be filled with mucilage (Fig. 1).

One of the most interesting observations noticed in this study was the large number and wide variation in size of vacuoles in the water storage mesophyll cells, and the occurrence of numerous vesicles within the vacuoles. The mesophyll cells contained many vacuoles (Fig. 2) which vary in size from small to rather very large, and they occupied a considerable portion of the cell volume. Tonoplast enclosing each vacuole was generally irregular in outline. All the vacuoles contained precipitates. Myelin-like figures, although irregular in outline (Fig. 3), and plasmalemmasomes (Fig. 4) were often observed in the vacuoles, especially near the peripheral cytoplasm. Sometimes they were found within the vacuole area close to the other organelles in the cytoplasm such as chloroplasts or microbodies or even with the nucleus. Vacuole-like spaces of membranous compartmentation within the vacuoles or bladder-like vesicles extending from the cytoplasm into the vacuole were frequently encountered, particularly in *O. malacophyllus* (Figs. 2, 5), a species which occurs along the shore where plants are exposed to sea water spray.

Other noticeable features were the presence of the tubular configurations found in the cytoplasm and separation of the plasmalemma from the cell walls. The thin cytoplasm distributed irregularly around the chloroplasts formed tubular conformations (Figs. 6~7), giving them a peculiar appearance. Numerous invaginations and undulations of the plasmalemma were also often detected in the mesophyll cells (Figs. 8~9). Some invaginations appeared like circlets or vesicles forming from the plasmalemma (Figs. 9~10).

The chloroplasts were distributed peripherally along the cell outline and their stroma were rather homogenous. They had well-developed thylakoid systems (Fig. 11), small plastoglobuli and occasionally some starch grains. More plastoglobuli were observed in the species with the thickest leaves, *O. japonicus* (Fig. 12), which exhibited the most mucilaginous nature among three species examined. No phytoferritin was observed within the chloroplasts. Microbodies were also common in the cytoplasm (Fig. 13), but crystalline inclusions have not been seen within the microbodies of any of the *Orostachys* examined. These microbodies were mostly in close association with chloroplasts. Dark-stained bodies were scattered in the cytoplasm of all *Orostachys* studied, but most abundantly in *O. sikokianus*.

Endoplasmic reticulum and dictyosomes were distributed along the extremely thin peripheral cytoplasm in most cases and detected more often in cells containing mucilage materials. Individual elements of the ER were scattered throughout the cytoplasm. Dictyosomes consisting of several cisternae with vesicles were also commonly observed (Fig. 14). Typical as well as highly branched plasmodesmata were discovered between the mesophyll cells. In particular, clusters of plasmodesmata were occasionally encountered between the mesophyll cells in places where cells seemed to contain mucilage materials. Irregularly branched plasmodesmata often left the two neighboring cells interconnected peculiarly, showing somewhat open lumens through the cell wall areas (Fig. 15).

DISCUSSION

Ultrastructural characteristics of the succulent mesophyll cells have been investigated. The mesophyll tissue appeared rather unspecialized in light microscope, since succulents are known to possess relatively high volumes of undifferentiated water-storing tissues and intercellular spaces (Glagoleva *et al* 1992). Ultrastructure of the CAM cells generally show the same nearly empty cells because of the dominating vacuoles. It can be seen that the peripheral cytoplasm surrounding the vacuole is extremely thin, giving the appearance of little space for any organelles. The cell vacuoles of CAM plants often appear filled with precipitates, such as those observed in the vacuoles of the cells. These are probably tannins or tannin-like substances (Kluge and Ting, 1978). Malate is also believed to be stored in these vacuoles (Homer and Homann, 1972). If the assimilating cells also have large storage vacuoles, CAM metabolism may be expected (Kluge and Ting, 1978). Membranous compartmentation within the vacuoles as observed in this study has been reported previously in other succulent species, such as *Mesembryanthemum crystallinum* (Willert and Kramer, 1972). However, *O. sikokianus* showed more compartmentation of various sizes extending farther into the cytoplasm than has been reported for other species. Also numerous bladder-like vesicles with darkly stained bodies extending from the cytoplasm into the vacuole were seen in the mesophyll cells of this species. When various sizes of vacuoles occurred in the cell, interrelationships and transitory changes between the small and large vacuoles has been suggested (Thomson and Journett, 1970). The myelin-like figures associated with modification of the plasmalemma are quite intriguing. Similar structures have been reported in a variety of other plants and these structures possibly represent accumulations of excess and turnover products of membrane materials (Thomson and Journett, 1970).

Double membrane-bounded plasmalemmasomes packed with short tubuli were often found within the vacuoles of the mesophyll cells neighboring the vascular cells. However, there is no clear suggestion whether those plasmalemmasomes perform any specific role in CAM of *Mesembryanthemum crystallinum* or any other succulent plants. In addition to this, the significance or role of the tubular configuration found in the cytoplasm of the mesophyll cells in species remains to be determined.

Regarding the space observed between the cell wall and plasmalemma of *Mesembryanthemum crystallinum*, Willert and Kramer (1972) claimed that, in the "deacidified phase" of CAM, with low malic acid content of the cell, the chloroplasts and the surrounding cytoplasm were attached to the cell wall. In contrast, during the "acidified phase" chloroplasts were detached from the wall, leaving a space between the plasmalemma and the wall. This space has been shown to be filled by fibrillar structures resembling the "Hechtsche Fäden" in plasmolyzed cells (Sitte, 1963). This structure was also reported in the study of *Bryophyllum species* (Kramer and Willert, 1972) and such space was interpreted as a vacuole-like compartment where malic acid could be stored. An assumption has been made that the occurrence of this vacuole-like space is presumed to be related to Crassulacean acid metabolism (Kramer and Willert, 1972). However, the possibility that it is a systematic artifact caused by the fixation of the tissue during EM preparation has not been ruled out (Kluge and Ting, 1978). The "Hechtsche Fäden" was not

observed in leaves studied. Numerous invaginations and undulations of cell plasmalemma were noticed. Many of the variations in the plasmalemma probably indicate that the plasmalemma is a highly active interface as indicated in the guard cell plasmalemma of the succulent *Opuntia* species (Thomson and Journett, 1970).

In a stem succulent cactus, *Echinocactus* species, numerous osmophilic plastoglobuli have been reported in the chloroplasts of the outer cortical tissue, whereas the chloroplasts of the inner cortex bodering the pith tissue had both starch and plastoglobuli (Thomson and Platt, 1973). In the present study, more plastoglobuli were detected in chloroplasts of the thickest leaved species, *O. malacophyllus*. This species also showed the most abundant mucilaginous substance, as reported in one of the leaf succulent *Portulaca* species with very thick leaves (Kim and Fisher, 1990). It was assumed that storage lipid may be reserved as a carbon reserve, and the lipids stored in the plastoglobuli might be linked to the starch pool (Thomson and Platt, 1973).

CAM plants perform photorespiration as do C3 plants. In this connection, the presence of microbodies as a structural precondition of photorespiration have been demonstrated in the photosynthesizing cells of *Kalanchoe daigremontiana* (Kapil *et al.*, 1975). Microbodies were also observed in association with chloroplasts in the stem of *Echinocactus* species (Thomson and Platt, 1973), as they were in the leaves of species in this study.

Electron micrographs of typical CAM leaves of *Crassula* (Langenheim and Thimann, 1982) and *Aloe* species (Homer and Homan, 1972) revealed similar chloroplast morphology as in species, exhibiting dense thylakoid systems, plastoglobuli and starch grains, but no phytoferritin structures in their chloroplasts. A well-developed thylakoid system within the chloroplasts indicates that the photosynthetic capacity of the water-storing mesophyll cells is probably active in these succulents. The foliar ultrastructure of species demonstrated many cellular structures similar to those of the other features reported previously from other species known to be CAM performing plants. This suggests the probable occurrence of the CAM mode in these species. However, an extensive ultrastructural investigation along with the appropriate physiological studies need to be carried out to confirm the precise structural CAM nature of these species.

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SUMMARY

Ultrastructural characteristics were examined with leaves of three species, *O. japonicus* A. Berger, *O. malacophyllus* Fisch., and O. *sikokianus* Owhi that probably have CAM mode. The mesophyll cells of these *Orostachys* possessed vacuoles with precipitates, myelin-like figures, and plasmalemmasomes, along with typical chloroplasts, microbodies and darkly stained bodies in their thin peripheral cytoplasm. Separation of the plasmalemma from the cell wall, leaving a space between them, was a common phenomenon in these species. A complex array of small to large vacuoles which contain small, membrane-bounded vesicles or vacuole-like structures

were frequently found. A well-developed thylakoid system was observed in the chloroplasts and this indicates that the photosynthetic capacity of these mesophyll cells is probably active. A peculiar configuration of cytoplasm, especially around the chloroplasts, was also encountered. The variety of cytoplasmic constituents and vacuoles suggest the water-storing mesophyll cells may be complex in function. Some cellular features detected in this study strongly suggest the possible occurrence of CAM mode in *Orostachys* species.

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FIGURE LEGENDS

Fig. 1. Peripherally arranged chloroplasts (C) and large intercellular space (IS) filled with mucilage materials (*) in *Orostachys sikokianus*. Bar=2 μm.

Fig. 2. Numerous vacuole-like compartments (Vc) of various sizes or vesicles in the vacuoles (V) in O. *malacophyllus*. Bar=2 μm.

Fig. 3. Myelin-like figures (M) in the vacuole of *O. malacophyllus*.
Note close association with the chloroplast and microbody (mb). Bar=0.5 μm.

Fig. 4. Double-membrane bounded plasmalemmasomes (arrow heads) found in the cells neighboring vascular tissue in *O. japonicus*. Bar=0.5 μm.

Fig. 5. Small vacuole or vacuole-like compartments within the large vacuole observed in *O. malacophyllus*. Notice two darkly-stained bodies (D) in the cytoplasm. Bar=1 μm.

Fig. 6. Tubular configuration (arrow heads) formed in the cytoplasm between the cell wall and chloroplast in *O. sikokianus*. Also notice the space (*) between the cytoplasm and cell wall and undulation of the plasmalemma in a cell below. Bar=0.3 μm.

Fig. 7. Tubular configurations observed in *O. japonicus*. St; Starch grain. Bar=1 μm.

Fig. 8. Vacuole-like space formed between the chloroplast and the cell wall in a cell (above) and plasmalemma separation in a cell (below) of *O. malacophyllus*. Bar=1 μm.

Fig. 9. Strong undulation giving wiggly appearance of the plasmalemma in *O. sikokianus*. Bar=2 μm.

Fig. 10. Plasmalemma invaginations and vacuole-like compartmentations detected in *O. japonicus*. cw= cell wall. Bar=0.3 μm.

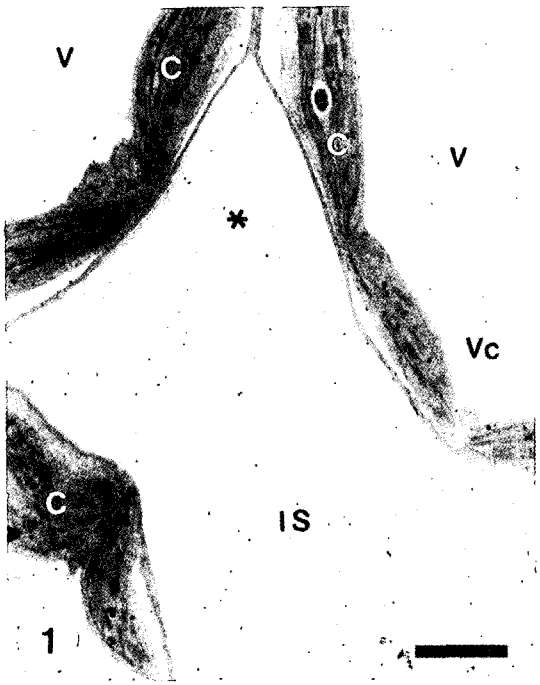
Fig. 11. A well-developed thylakoid system (th) with homogenous stroma (S) in a chloroplast of *O. japonicus*. Ce=chloroplast envelope. Bar=0.1 μm.

Fig. 12. Numerous plastoglobuli appearing as dark circular bodies in *O. japonicus*. Bar=1 μm.

Fig. 13. Four microbodies closely associated with chloroplasts in *O. malacophyllus*. Bar=0.5 μm.

Fig. 14. Endoplasmic reticulum (er) and dictyosome (d) within the thin, peripheral cytoplasm (Cy) of *O. malacophyllus*. Bar=0.5 μm.

Fig. 15. Cell wall area showing typical (arrows) and branched plasmodesmata (arrow heads) of *O. japonicus*. Bar=0.3 μm.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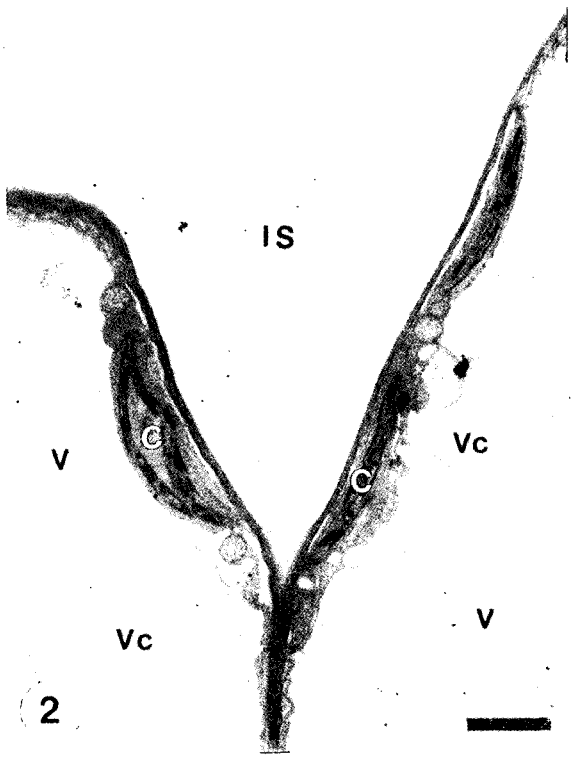
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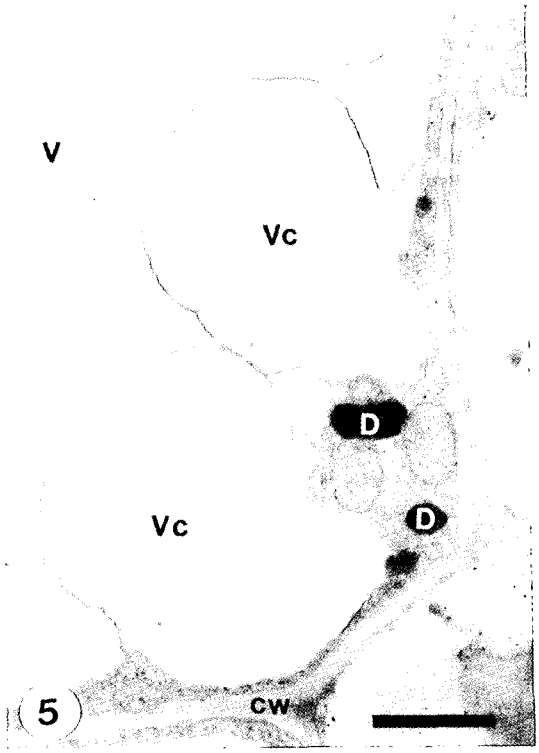
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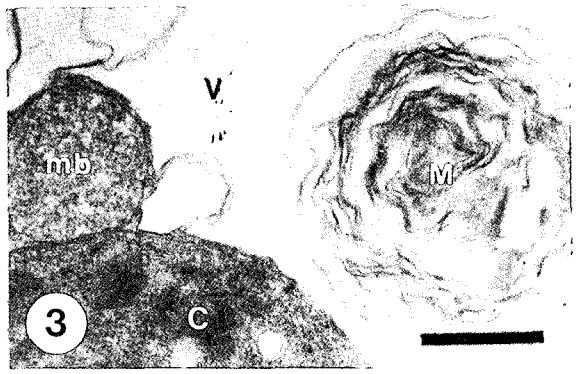
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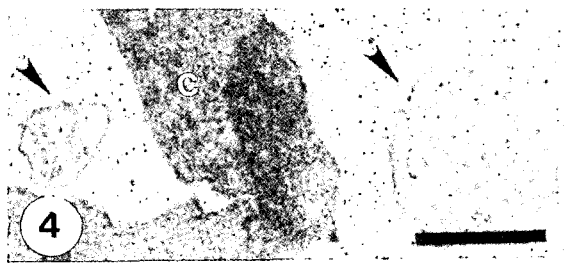
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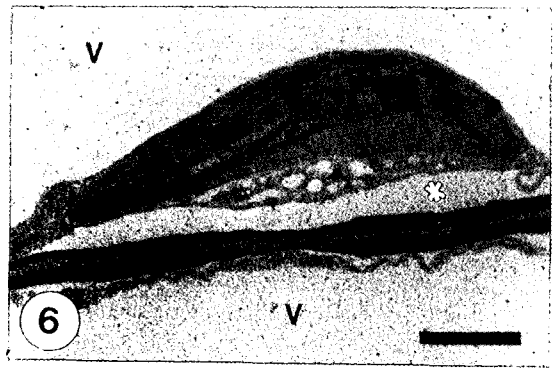
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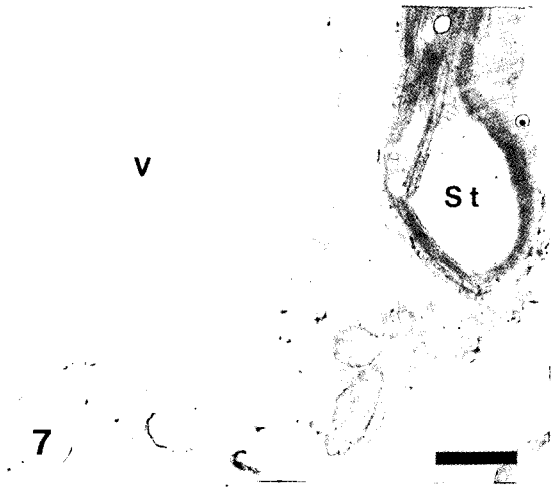
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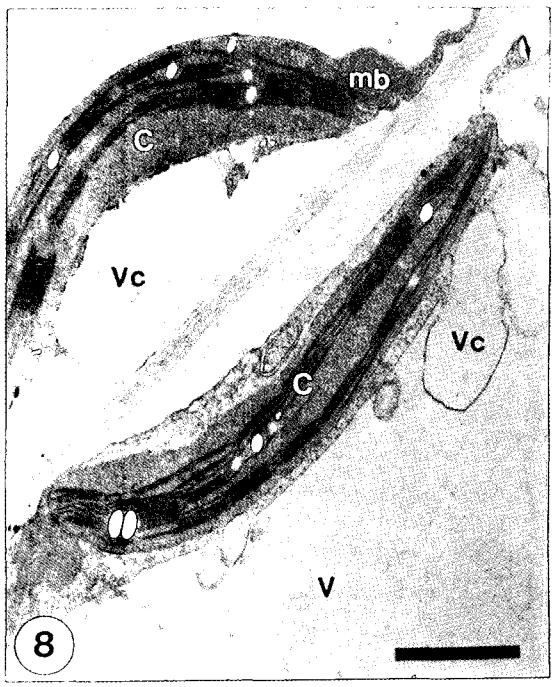
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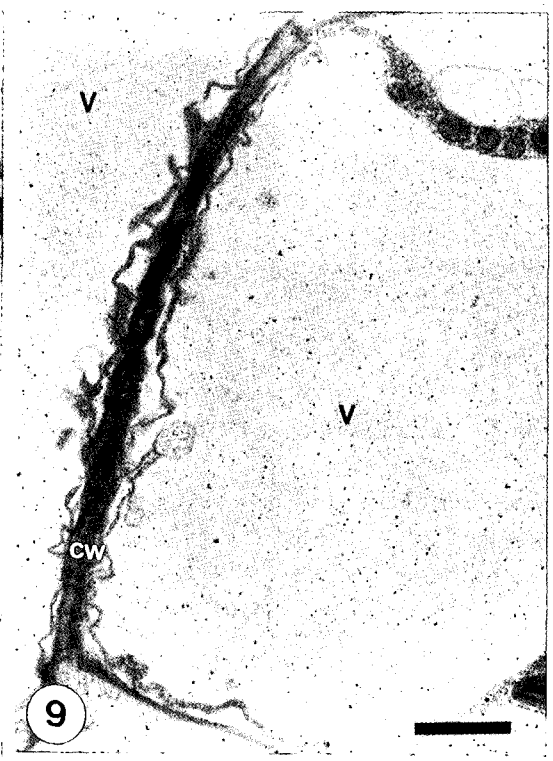
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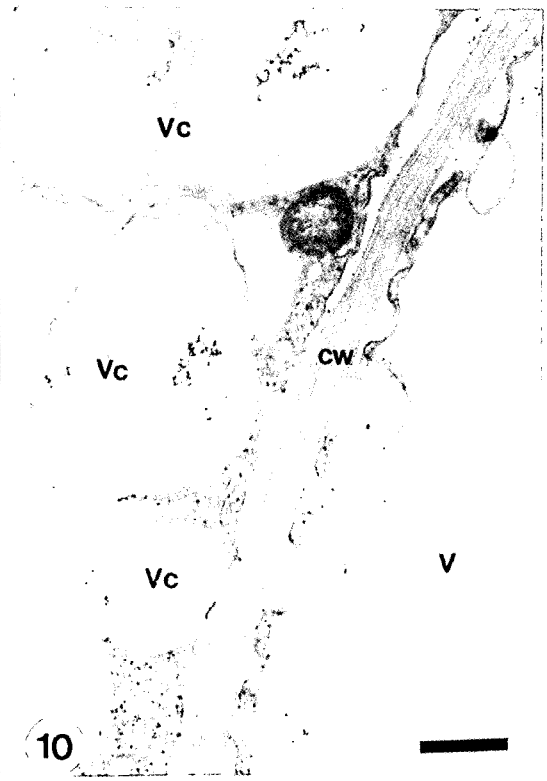
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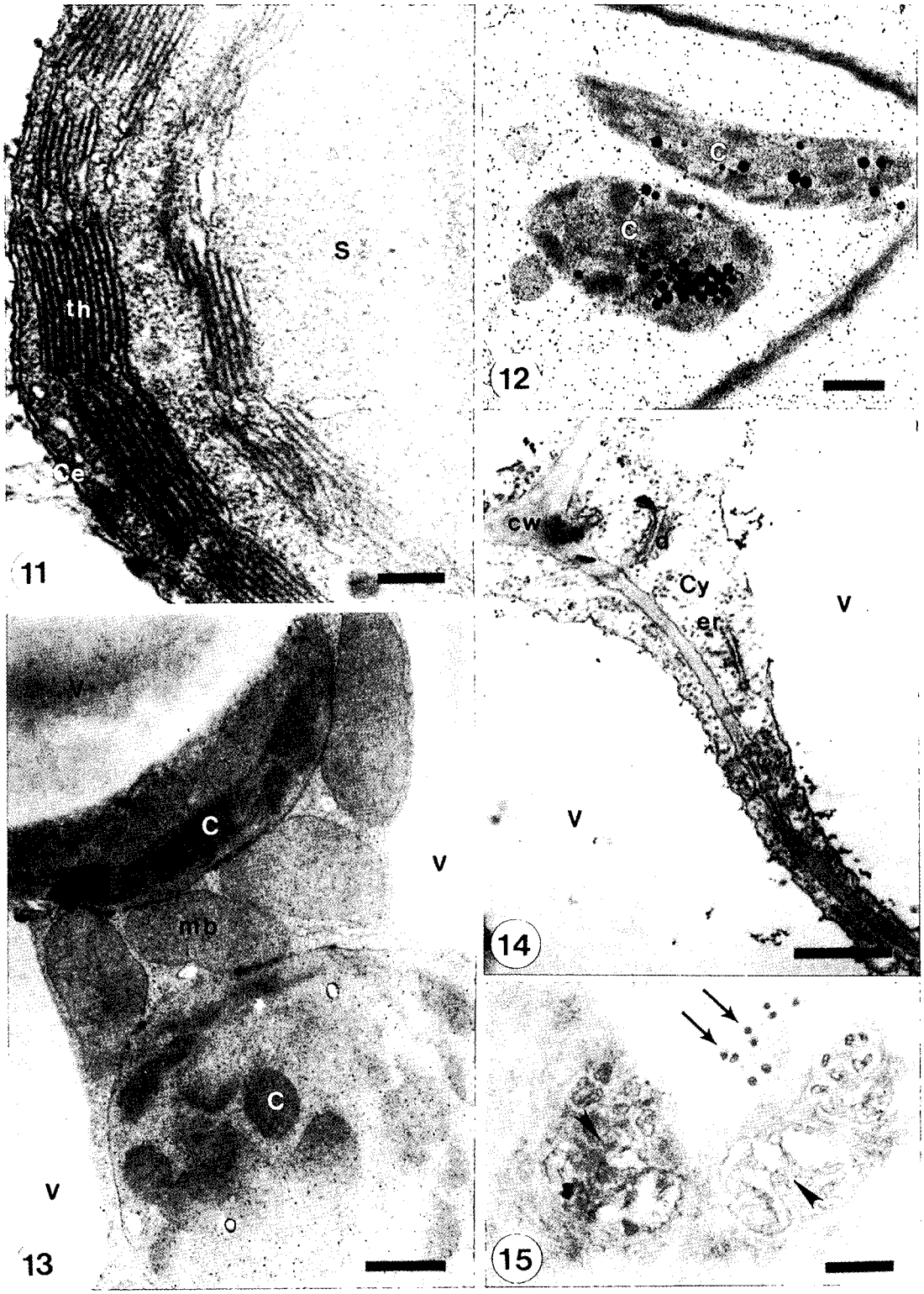
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