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Structural Differentiation of Photosynthetic Tissue in Kranz Anatomy of *Salsola* Species

In Sun Kim
Biology Department, Keimyung University, Taegu 704-701, Korea
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*Salsola*속 Kranz구조내 광합성조직의 구조분화

김 인 선
계명대학교 자연과학대학 생물학과

ABSTRACT

Leaves of two developmental stages of *Salsola* species, young and mature, were examined to reveal the structural and functional relationships in the photosynthetic tissue using anatomical and ultrastructural criteria. Both young and mature leaves had Kranz anatomy of the Salsolid type with two layers of chlorenchyma on the leaf periphery: an outer layer of palisade mesophyll cells and an inner layer of compact bundle sheath cells with centripetally arranged organelles. The chlorenchyma was continuous in young leaves, while it was discontinuous in mature leaves. The main vascular bundle occupied the central position in the leaf, but the small peripheral vascular bundles were in contact with the chlorenchyma. Structural dimorphism of chloroplasts was obvious in bundle sheath cells of mature leaves exhibiting noticeable grana reduction, whereas mesophyll cell chloroplasts had well developed grana in all cases. Plasmodesmata were less numerous and rather simple in young leaves relative to well-developed secondary plasmodesmata of the later stage. According to the current data, features of two stages of *Salsola* leaves corresponded to NADP-ME biochemical subtype on the basis of photosynthetic cell ultrastructure. Implications of developing such anatomical and ultrastructural data of *Salsola* species and biochemical characteristics reported in other C-4 species have been discussed.

Key words : Kranz anatomy, Leaf development, *Salsola*, Structural differentiation

INTRODUCTION

The two photosynthetic cell types, bundle sheath and mesophyll cells, in Kranz structure is essential for the efficient operation of the C-4 photosynthetic pathway (Hatch, 1987). In addition to the standard Kranz type leaf anatomy, some C-4 species exhibit unusual features

* Correspondence should be addressed to Dr. In Sun Kim, Biology Department, Keimyung University, Taegu 704-701, Korea. Ph: (053) 580-5305, FAX: (053) 580-5164, E-mail : botany@kmu.ac.kr

(Voznesenskaya & Gamaley, 1986; Kim & Fisher, 1990). The specialized leaf anatomy found in C-4 species of chenopods is very distinctive. The leaf structure having two distinct cell layers of photosynthetic tissue, the chlorenchyma, located between the epidermis and central water storge tissue probably deviated from the typical bundle sheath Kranz type anatomy (Voznesenskaya & Gamaley, 1986; Dengler et al., 1995). The chenopods have evolved a number of very different C-4 structures ranging from typical bundle sheath arrangements in the genus *Atriplx* (Carolin et al., 1975, 1978; Dengler et al., 1995) to cylindrical chlorenchyma sheaths surrounding internal water storage tissue in the leaf succulent genera *Salsola* (Carolin et al., 1975; Denger & Nelson, 1999; Kim, 2001). These different anatomies have been known to correspond with different biochemical pathways (Dengler et al., 1995; Pyankov et al., 1997, 1999).

Four structural types of Kranz anatomy have been recognized among C-4 species of the chenopods (Carolin et al., 1975, 1978; Dengler & Nelson, 1999). In general, C-4 plants are divided into three biochemical subtypes, the NADP-malic enzyme (NADP-ME) type, the NAD-nalic enzyme (NAD-ME) type, and the phosphoenolpyruvate carboxykinase (PCK) type, depending on the nature of the decarboxylation process (Hatch, 1987; Kanai and Edwards, 1999). These three biochemical types are associated with distinct structural features of the bundle sheath cells (Edwards and Walker, 1983; Hatch, 1987; Denger & Nelson, 1999). The majority of *Salsola* species have C-4 type photosynthesis (Carolin et al., 1975; Shomer-Ilan et al., 1981; Pyankov et al., 1997). The leaves of C-4 *Salsola* species are succulent and cylindrical with Salsoloid type anatomy (Carolin et al., 1975). They have a centric structure with a centrally located main vascular bundle, which is surrounded by water-storage parenchyma in the center and compact inner bundle sheath and outer mesophyll cells on the periphery. Considerable diversity in life form, and in structural and biochemical features of photosynthesis occur in this genus (Pyankov et al., 1999). C-4 *Salsola* species can be subdivided, on the basis of ultrastructure, into two groups which are classified biochemically as NAD- or NADP-ME subtypes (Glagoleva et al. 1992; Pyankov et al., 1997, 1999).

There is some information on the diversity of photosynthetic tissues in C-4 species, but less is known about their development in *Salsola* leaves. Comparative studies of photosynthetic tissue development in leaves of C-4 species contribute significantly to our understanding of the mechanism of C-4 photosynthesis. However, such studies in leaves of C-4 chenopods are limited except for Atriplex (Dengler et al., 1995). The present study was carried out to characterize the photosynthetic tissue development primarily in leaves of *Salsola komarovii*, a well-known C-4 chenopod (Voznesenskaya & Gamaley, 1986). The leaf tissue used for this characterization was also used in a parallel ultrastructural study for the temporal coordination of developmental changes in cell structures.

MATERIALS AND METHODS

Plants : Approximately 5~7 mature plants were collected from sandy soils along the coastline in ByunsanBando, Cheonbuk, Korea. After they were transferred to the laboratory, leaf samples were taken from the plants and immediately used for the experiment. Young leaves of 3~4 mm long and mature leaves of >15 mm were used for the following study. Fixation of leaf samples for light microscopy (LM) and transmission electron microscopy (TEM) was carried out in the morning to avoid accumulation of starches in chloroplasts.

LM, TEM, SEM : Both for LM and TEM, ca. 1~2 mm^2 leaf sections sliced from the median portion were fixed in a 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 3 hours at room temperature and postfixed in 2% OsO_4 overnight at 4°C. Then, samples were

dehydrated through graded acetone series and embedded in Spurr low-viscosity resin. Ca. 0.8~1.0 μm thick and 60~90 nm ultra-thin sections were made with the Ultracut-S ultramicrotome using histo- and diamond knives. respectively, 0.35% chloroform-diethanol formvar coated grids were used for the ultra-thin sections. Thick sections were stained with 0.5% toluidine blue O for 30 seconds and examined by Zeiss Zenalumar microscope. Ultra-thin sections were stained with 2% uranyl acetate and 1% lead citrate for 30~45 minutes each and studied with Hitachi-H 7100 TEM at Korea Basic Science Institute (KBSI) Taegu Branch. For scanning electron microscopy (SEM), leaves were processed as same as the TEM fixation and dehydration, but substituted by isoamyl acetate after dehydration, then further processed to critical point drying and sputter coating before examination with Hitachi-S 4200 at KBSI Taegu Branch.

RESULTS

Leaf growth – leaves initially exhibited a vertical orientation as they extended from the shoot apex, and tissues at the apex became a spine and the photosynthetic tissue differentiated downward. In all portions of the leaf, the water storage cells developed first and further in the order of the central vein, the photosynthetic tissue, and the smaller peripheral veins. The latter was embedded in the water storage cells and close to inner chlorenchymatous sheath cells, often showing direct contact with the photosynthetic tissue layer. Leaf areas sampled for this study were clearly associated with central and peripheral veins, while only some of the peripheral veins and central vein can be distinguished in apical regions of young leaves.

Bundle sheath and mesophyll cell differentiation – At the onset of the leaf development, only a rudimentary lamina with dividing ground mesophyll cells was present. But the meristematic cells beneath the epidermis divided rapidly to form 2~3 continuous photosynthetic layers just outside of where smaller veins developed in young leaves. Most of divisions within the peripheral mesophyll gave rise to the bundle sheath and mesophyll cell layers (Fig. 1). In the region sampled from young leaves, bundle sheath and mesophyll cells appeared less distinct in shape and size. The two layers of cells in the mature leaves became discontinuous, while forming conspicuous mesophyll cell layer directly adjacent to the bundle sheath layer and the larger water storage cells in the center. During the later part of leaf expansion, both bundle sheath and mesophyll cells increased in size and the anatomy, morphology, and ultrastructure were clearly differentiated into the mature forms (Figs. 2-3). As mentioned above, the size of mesophyll and bundle sheath cells were not significantly different from each other in young leaves (Fig. 4), but they were distinguished as the cells expanded. Dense cytoplasm including numerous coated vesicles and Golgi bodies (Figs. 5-6) was the noticeable feature in early development, while the mature leaves showed a fully developed succulence with the mesophyll cell well differentiated into a peripheral chlorenchyma and central water storage tissue. With maturation, mesophyll cells elongated and conspicuously vacuolated with peripheral chloroplasts, while in the bundle sheath cells vacuoles occupied a smaller proportion of cell volume and chloroplasts were asymmetrically placed toward the vascular tissue (Fig. 7).

The inner chlorenchymatous sheath was the distinguishing feature of the Salsoloid C-4 anatomy. The chloroplasts of the sheath cells were either centripetally aligned or occupied most of the cell volume. The bundle sheath chloroplasts exhibited reduced development of grana with relatively few lamellae in each granum. Rudimentary to agranal and single thylakoids were numerous. On the other hand, the mesophyll cells had huge vacuoles and organelles were distributed along the side walls. They had normal chloroplasts with well-developed grana and numerous lipid droplets. The

crystalline inclusion body (Fig. 8) was found in the mesophyll chloroplasts of both young and mature leaves. However, it was not found in the bundle sheath cell chloroplasts. A pronounced form of plasmodesmata development was detected between the two neighboring cells in the photosynthetic tissue. Rather simple and slightly more plasmodesmata were formed in the walls between the bundle sheath and mesophyll cells of the young leaves (Fig. 9), whereas prominent and abundant plasmodesmata occurred in the two cell type interfaces. They were concentrated in the primary pit field of thin wall areas (Fig. 10). However, plasmodesmata were less frequent in thick walls of bundle sheath cell interface and in thin walls of adjacent mesophyll cells. Plasmodesmatal connections at the bundle sheath-mesophyll interfaces were highest (Fig. 11) than any other wall interfaces, indicating bundle sheath cell-mesophyll routes as the major symplastic solute pathway in *Salsola* species.

DISCUSSION

Most species of the genus *Salsola* which have been examined to date exhibit C-4 photosynthesis in their leaves (Dengler & Nelson, 1999; Voznesenskaya et al., 1999). The leaves of C-4 *Salsola* species are succulent and cylindric with mostly Salsoloid type Kranz anatomy (Carolin et al., 1975; Vozensenskaya & Gamaley, 1986; Akhani et al., 1997; Dengler & Nelson, 1999; Pyankov et al., 1997, 1999). The foliar mesophyll tissue differentiated into three distinct layers: a layer of palisade parenchyma below the epidermis, an inner chlorenchymatous sheath, and central water storage tissue containing vascular bundles. The inner chlorenchymatous sheath is considered as the distinguishing feature of the Salsoloid C-4 anatomy. All anatomical and ultrastructural measurements on *Salsola* examined in this study also demonstrated features of the C-4 photosynthesis in their leaves. In *Salsola komarovii*, leaves showed the lower degree of grana development in bundle sheath cell chloroplasts and appearance of only a few small mitochondria in bundle sheath cells of Kranz tissue that are characteristec of NADP-ME type of C-4 photosynthesis (Hatch, 1987; Dengler & Nelson, 1999). The wall in the two cell type interfaces in NADP-ME subtype is generally thicker and denser than those in other subtypes (Dengler & Nelson, 1999).

Immunolocalization study of the bundle sheath cell-specific enzyme, RuBPCase, and of the mesophyll cell-species enzyme, PEPCase has reported the development of the C-4 pattern of photosynthetic enzyme expression during *Atriplex* leaf growth (Dengler et al., 1995). In *Atriplex*, the earliest appearance of RuBPCase follows the formation of bundle sheath cell precursors and the tissue-specific accumulation of RuBPCase begins as soon as the bundle sheath and mesophyll cells are delimitated (Dengler et al., 1995). However, a different pattern is known in two different sizes of leaves in *Amaranthus* (Wang et al., 1992). According to Wang et al. (1992), it was found that RuBPCase was expressed in both bundle sheath and mesophyll tissue at the first stage, but disappeared from mesophyll by later stage. Expression of the two photosynthetic enzymes are known to precede the divergence in cell ultrastructure such as bundle sheath cell and mesophyll cell size, the number and size of chloroplasts, chloroplast starch and thylakoid stacking, and cell wall thickness (Dengler et al., 1995). With the exception of the mesophyll-specific features, RuBPCase generally occur at a greater rate and continue longer activity than PEPCase in mesophyll cells (Dengler et al., 1995). Thus, the establishment of the C-4 pattern of RuBPCase and PEPCase expression in *Salsola* species needs to be investigated to follow and reveal the biochemical characteristics of their expression pattern during its photosynthetic tissue differentiation.

It seems clear that *Salsola komarovii* has all the characteristics constituting the C-4 photosynthesis. The anatomical specialization is differentiated into a chlo-

renchymatous sheath, either continuous or discontinuous, surrounding the central structures. The current findings mostly correspond to one of the C-4 biochemical subtypes, namely the NADP-ME type. The spatial differentiation of the chlorenchyma is what probably contributed to the structural and functional advantages for an adaptation. Unlike such assumption, however, any positional relationships in the C-4 development of some species having modified Kranz anatomy as in *Salsola komarovii* can not be clearly explained. Thus, if comparable analyses of the temporal and spatial patterns of developmental stages in other C-4 *Salsola* are carried out, meaningful comparisons of the unusual C-4 photosynthetic tissue differentiation will be revealed. Immunolocalization of RuBPCase and of PEPCase expression pattern during *Salsola komarovii* leaf development will be the subject of a subsequent report.

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<국문초록>

Salsoloid Kranz 구조를 지닌 다육질성의 *Salsola komarovii*의 두 단계 잎의 광합성 엽육조직에 대하여 LM

및 TEM에 의한 구조적 분화발달 양상을 연구하였다. 수분저장조직을 둘러싸는 내부 유관속세포층 및 외부 엽육세포층으로 구성된 두 층의 광합성조직은 어린 잎에서는 연결되어 발달하나 성숙한 잎에서는 불연속적으로 발달하였다. 중앙의 유관속 외 다수의 미세맥들은 대개 내부 유관속세포층과 접하여 형성되며 발달초기에는 이 두 세포층의 구조적 차이는 뚜렷하지 않았다. 이들 엽육조직이 신장 발달하면 Kranz 구조 내 내부 유관속세포층과 외부 엽육세포층은 세포형태, 세포소기관의 분포, 엽록체의 내부 특성, 세포벽의 비후, 원형질연락사의 발달양상 등에서 현저한 차이를 보이며 구조적으로 분화하였다. 특히 이들이 지닌 엽록체의 미세구조 및 원형질연락사 등의 특성은 생화학적으로 알려진 NADP-ME 유형과 거의 일치하였다. 이들은 세포특이성을 나타내는 C-4 광합성 효소의 발현양상을 조사하는 세포 면역화학법과 접목되어 연구될 것이다.

FIGURE LEGENDS

Abbreviations: BS, bundle sheath cell; C, chloroplast; CW, cell wall; E, epidermis; G, Golgi body; M, mesophyll cell; MT, mitochondria; V, vacuole; W, water storage cell. All TEM, except Figs. 1-3

Fig. 1. Cross section of the young leaf exhibiting compact, undifferentiated bundle sheath and mesophyll layers. Arrows indicate small peripheral veins. LM. Scale = 0.1 mm.

Fig. 2. Cross section of the mature leaf showing two distinct chlorenchyma layers. LM. Scale = 0.1 mm.

Fig. 3. Foliar morphology, in part, from fully expanded leaf. Arrowheads and arrows indicate marginal spines and stomata, respectively. SEM. Scale = 90 μm.

Fig. 4. Immature bundle sheath and mesophyll cells demonstrating no significant cellular differentiation between the two cell types. Young leaf. Scale = 10 μm.

Fig. 5. Numerous coated vesicles (arrowheads) and microtubules (arrows) in the mesophyll cell. Young leaf. Scale = 0.25 μm.

Fig. 6. Part of dense mesophyll cytoplasm with mitochondria and several Golgi bodies. Young leaf. Scale = 0.5 μm.

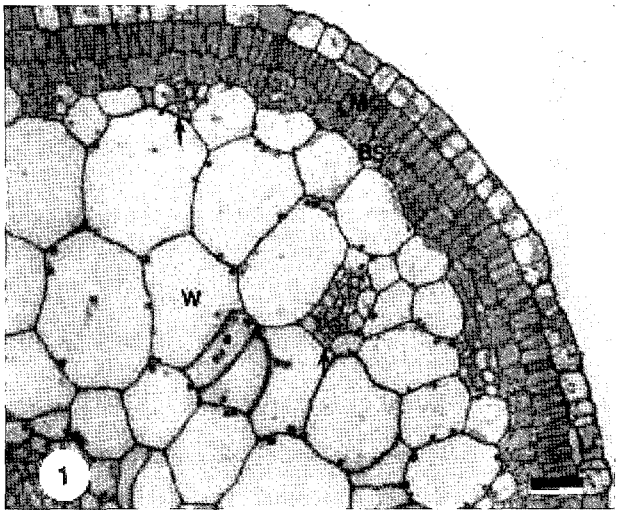
Fig. 7. Bundle sheath cells with centripetally arranged organelles and mesophyll cells with peripherally displaced chloroplasts. Note hugh vacuoles in the latter. Mature leaf. Scale = 4.0 μm.

Fig. 8. Crystalline inclusion body (arrowhead) within the mesophyll chloroplast. Mature leaf. Scale = 1.0 μm.

Fig. 9. Simple plasmodesmata (arrows) in the bundle sheath-mesophyll cell interfaces. Transverse view of plasmodesmata in the right bottom. Young leaf. Scale = 10 μm.

Fig. 10. Plasmodesmata (arrowheads) formed in the primary pit field of thin walls. Scale = 2.0 μm.

Fig. 11. Numerous secondary plamodesmata (arrows) developed in the walls between the bundle sheath and mesophyll cell. Mature leaf. Scale = 1.0 μm.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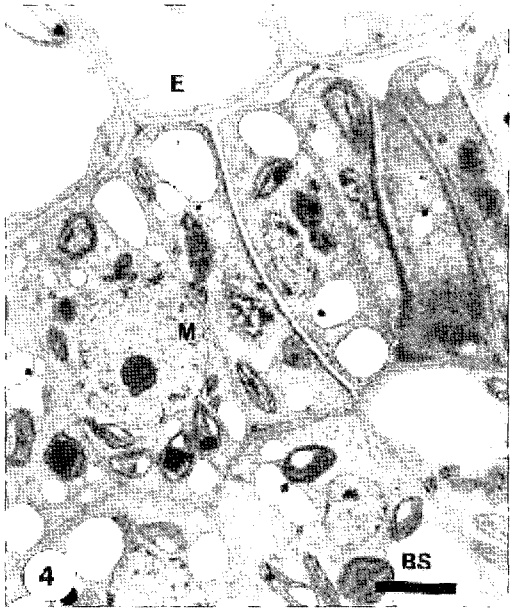
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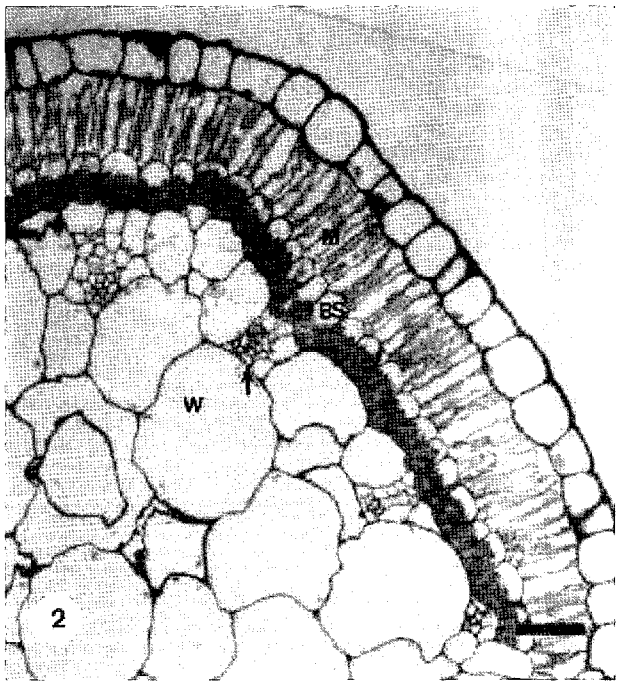
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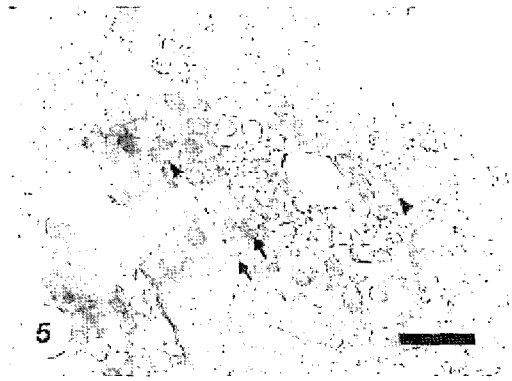
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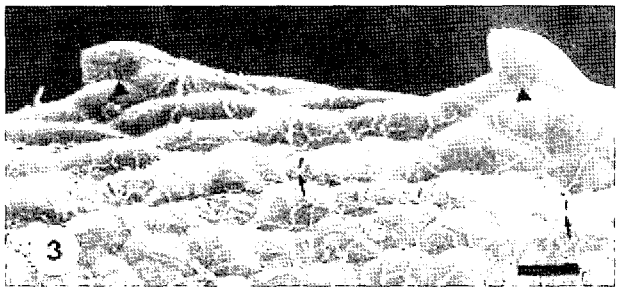
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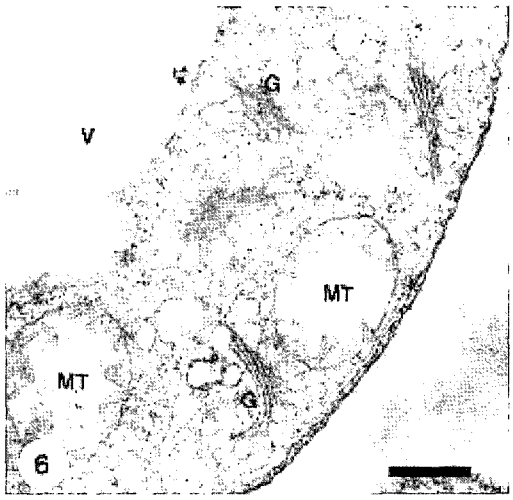
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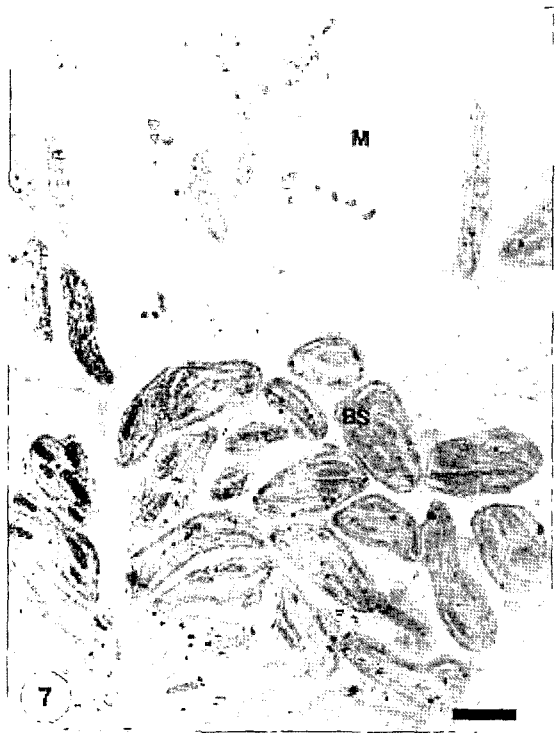
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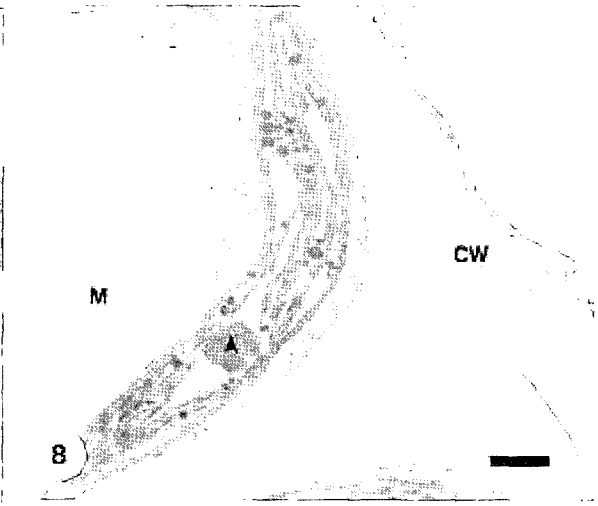
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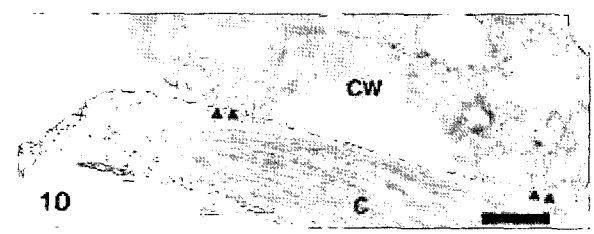
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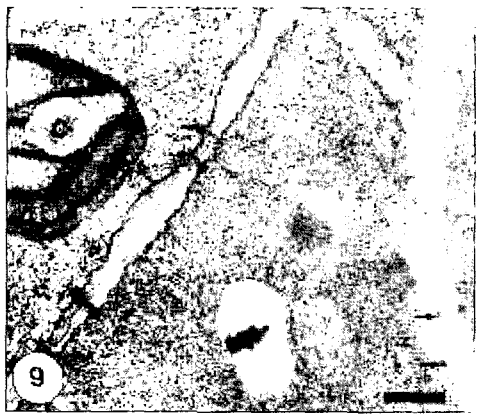
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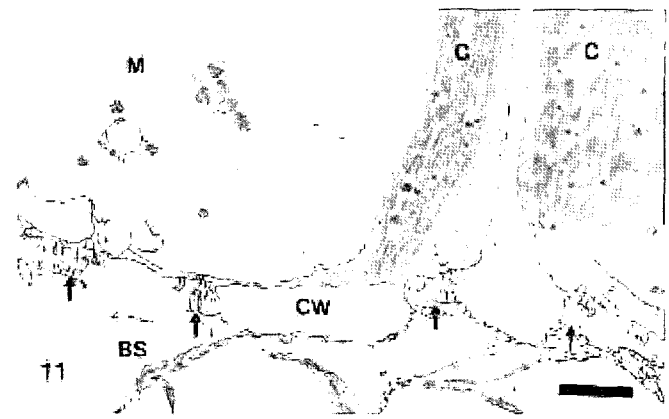
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