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Ultrastructure of the Adventitious Root Meristem and Callus Induced by Tissue Culture of Tobacco(*Nicotiana tabacum*)Leaves

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담배잎의 기내 培養에서 誘起된 부정근 분열조직 및 캘러스 세포의 微細構造

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ABSTRACT : Structures of the adventitious root meristem induced from callus culture of tobaco (*Nicotiana tabacum* cv. NC 82) leaves were investigated by light and transmission electron microscopy. Structural differences between *in vivo* root and callus cells were also examined by the microscopy. The submicroscopic features of the *in vitro* root cells were as follows. Intercellular spaces were not developed and nuclei with two nucleoli were observed occasionally. Plasmodesmata were found in groups or singly on transverse and longitudinal walls. Amyloplast solely filled with starch grains, with one to five electron - dense bands, was surrounded by single membrane. in the callus cells, vacuolization of central part in the cytoplasm having mitochondria with swollen cristae and starch grains like those of *in vitro* root cells was a distinct feature. Vesicles which were found between cell wall and plasma membrane may be arisen by a process of protoplasmic invagination. By comparing of ultrastructures between the cells of callus and *in vitro* root, we found that the distinct differences lied on thickened cell wasIls and hypertrophed vacuoles in the former, and less thickened cell walls and several small vacuoles in the later.

Key words : adventitious root, starch grains, vacuole, mitochondria

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Several theories explaining the direction and plane of cell division on differentiation were suggested to reveal the orgainization of the root system. Afterwards, ultrastructural studies on the organization and differentiation has been reported by several researchers (Clowes and Juniper, 1964 ; Northcote and Pickett-Heaps 1966 ; Morrë *et al.*, 1967 ; Chilvers, 1968 ; Juniper and French, 1970 ; Phillips and Torrey, 1974a ; Peterson and Vermeer, 1980). However, most of them describe the structure of root initials and root cap in relation to their fuction. Anatomical studies of the overall root meristem were restricted to the formation of the vacuole (Buvat and Robert, 1979), microbodies (Frederick *et al.*, 1968), differentiation of nucleolus (Hyde, 1967), and ultrastructure of root caps (Phillips and Torrey, 1974b).

Most of the fine structures of the plant cell were published after 1956. Two new branches of plant cell biology, ultrastructural cytophysiology and ultrastructural cytopathology, are focused on the reseach of the form in relation to function. Until now, however, the techniques of *in vitro* cultivation of plants have profited very little from ultrastructure studies. except for studies of protoplast culture. Tobacco plant is the model system of tissue and cell culture. Murashige and Nakano (1965) investigated the morphological behavior of tobacco calli. With regard to senescence in tissue explants, Bornman (1974) investigated plant cells cultured *in vitro* in particular. But ultrastructure of the adventitious roots originated from callus of tobacco cuture is not known. Therefore, we describe ultrastructural changes which occur in the adventitious root during rhizogenesis in tobacco culture.

MATERIALS AND METHODS

Induction of the callus and adventius roots.

The leaf segments of tobacco(*Nicotiana tabacum* cv. NC 82) were surface sterilized in 2% sodium hypochlorite solution, washed 5 times with sterile water, and cut by 5×5 mm to plate on Murashige and Skoog's basal salt mixture containing 100 mg/l inositol, 3% sucrose and 0.8% agar supplemented with plant hormones of 3.0 mg/l 2, 4-D and 2.0 mg/l kinetin (pH 5.8). The calli were induced 1 week after cuture in darkness at 25± 2 ℃. At the 30 days after culture, adventious roots were emerged from the lower parts of calli on the same media in the 16-hr a day light condition.

Sample preparation.

Samples for transmission electon microscopy (TEM) were prepared from the adventitious root by cutting the tissue of 3 mm from the tip. Root tip samples were fixed in a combination of 0.1 M paraformaldehyde plus 0.1 M glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 4 ℃ for 2 hr. The tissue was washed in the phosphate buffer four times for a total of 90 min and post-fixed in 0.05 M phosphate buffered 1 % OsO_4 at 4 a16* for 2 hr. Specimens were dehydrated in a ethanol series and embedded in Epon-Araldite mixture. For light microscopy, thick sections (ca. 1 μm) of epoxy-embeded tissue were cut and stained with 0.05% toluidine blue in the phosphate buffer. For TEM, thin sections of silver acetate and lead citrate, and examined at 80 kV in a JEM-100CX II electron microscope. Intact young roots were treated with same procedures for comparison. The callus tissues were excised as smalll blocks from the lower parts of the callus and performed the same fixation and staining procedure as above.

RESULTS

Initiation of the adventious roots.

Several roots were emerged endogenously from the lower parts of calli to reach 5 mm length after 35 days of culture and penetrated into the solid medium. They were relatively plump with yellow color, and had no lateral roots(Fig. 1).

Light microscopy of adventitious root.

Light microscopy of the longitudial sections of the meristematic region showed the quiescent center (QC) in *in vitro* root like the primary root, and it was a inverse T-shaped. Just beneath the QC, the central region crowded with many small cells indicated the occurrence of vigorous cell divisions. These small cells were characterized by many starch grains distinguishable from other starch grains. Most of the cortical cells were characterized by the deficiency of starch grians and their sizes larger than central cells. We could not observe columella in adventious roots (Fig. 2).

Anatomy by TEM

in vitro root

The nuclei were typically spherical or elliptical, which were positioned in the central part of the cell. Most of the nuclei had single nucleolus but some had two nucleoli (Fig. 3). Mitochondria with swollen cristae were round-shaped or elongated (Fig. 3, 4) in shape. The most striking feature of these cells was the attribute of starch grains in amyloplasts. Starch grains were singly or in cluster of 3-5 ones. They were distributed near a nucleus and two types were distinct. One type was chracterized by having highly electron-dense bands, some of them were even branched. The other type which occurred less than banded one was even branched. The other type which occurred less than banded one was even branched. The other type which occurred less than banded one was non-banded one. The latter type is a typical strucutre in ordinary starch grains (Fig. 5). The plasmodesmata were positioned along the transverse and longitudinal wall, but intercellular spaces are not found (Fig. 3).

Callus cell(*in vitro*)

There were two cell types in the callus tissues of tobacco (Figs. 6, 7). In one type, cytoplasm was thin and cell organelles were compressed along the cell wall by a large central vacuole (vacuolar type). The other type was characterized by its absence of a central vaculole(cytoplamic type). In the vacuolar type, intercellular spaces and cell walls were more prominent than the cytoplasmic type. Intercellular spaces were impregnated with electron-dense materials in the former (Fig. 6). But in the cytoplasmic type some intercellular spaces were not electron-dense (Fig. 6). There were no differences in the shape or size of cell organelles such as stach grains, mitochondia, or endoplasmic reticulum. We could observe not only a few swollen cristae in mitochondria and single file of rough endoplasmic reticulum but amloplasts filled with solely banded starch grains like *in vitro* roots. Several small vesicles are formed by invagination of a plasma membrane (Figs. 6, 10). The nucleus in the vacuolar type was ellipsoidal compared with sperical in cytoplasmic type. (Figs. 7, 8). A microbody between mitochondria contains a large rectangular crystallin structure (Figs. 7, 9).

in vivo root

The cells of root tip were long cells filled with cytoplasm scarecely vacuolated. They had homogenously thickened primary cell walls (Fig. 11). A spherical central nucleus was large with heterochromatin but the nuclear lamina was not distinct inside and adjacent to the inner membrane of nuclear envelope (Figs. 12, 13, 14). Some electron dense bodies and vesicles were found in small vacuoles (Fig. 12). Various spherical, dumbell-shaped mitochondria were distributed evenly along the cell wall (Fig. 12). We could not find stach grains like callus cells or adventitious roots.

DISCUSSION

The adeventitious root meristem originated endogenously from callus cells showed T-shaped and more or less the open type. Phillips and Torrey(1974 b) reported two types of starch grains in a amyloplast

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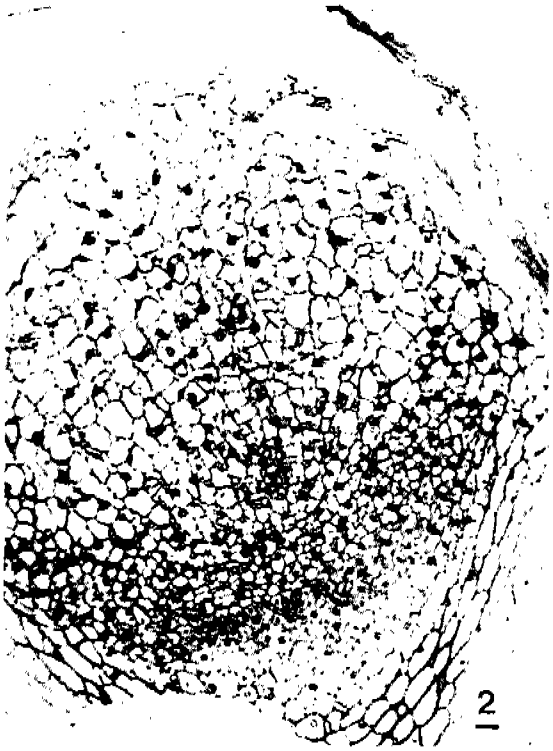

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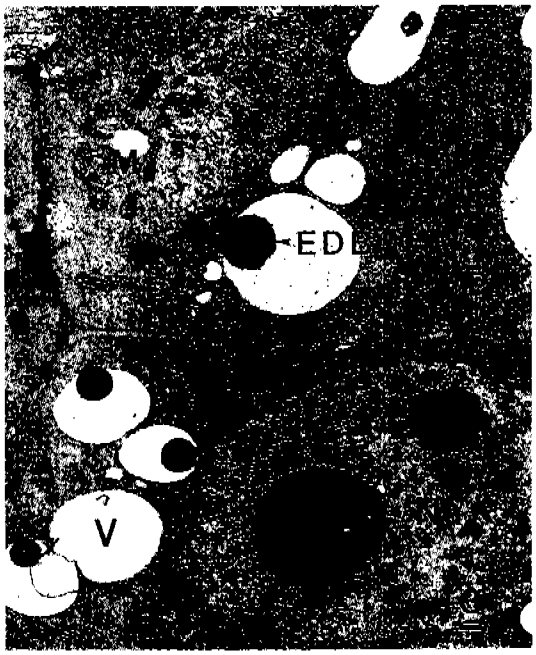

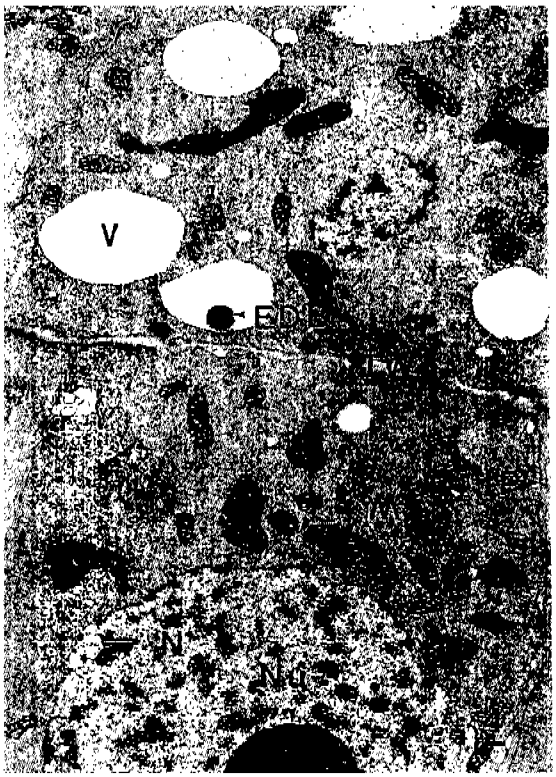

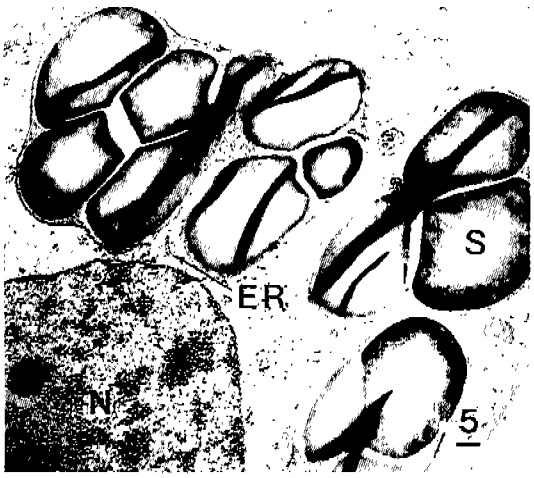

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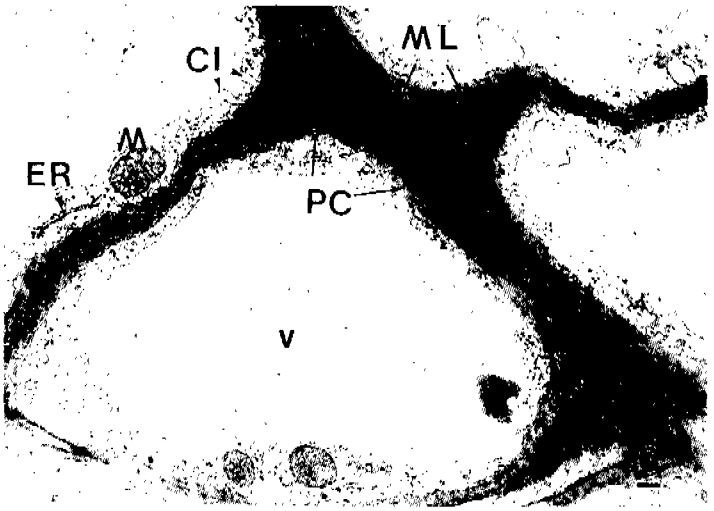
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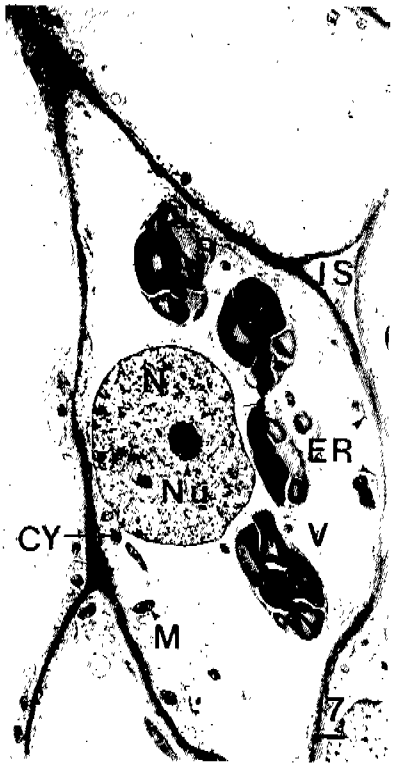
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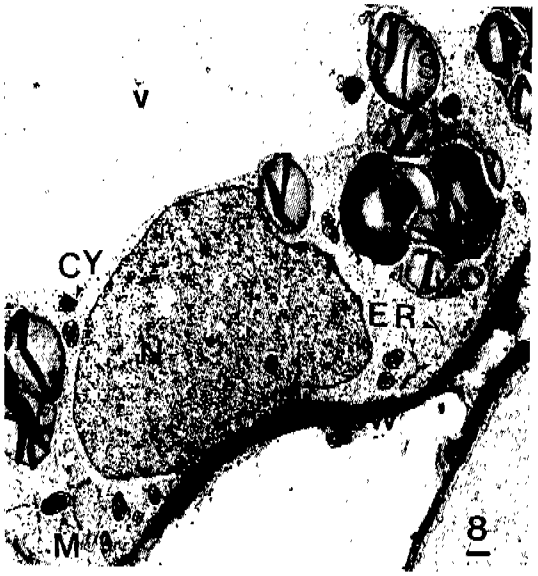
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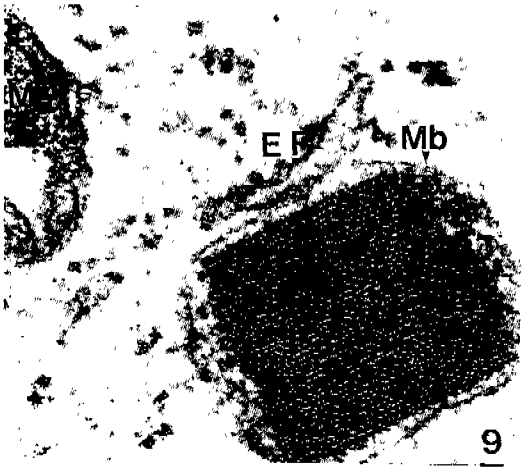
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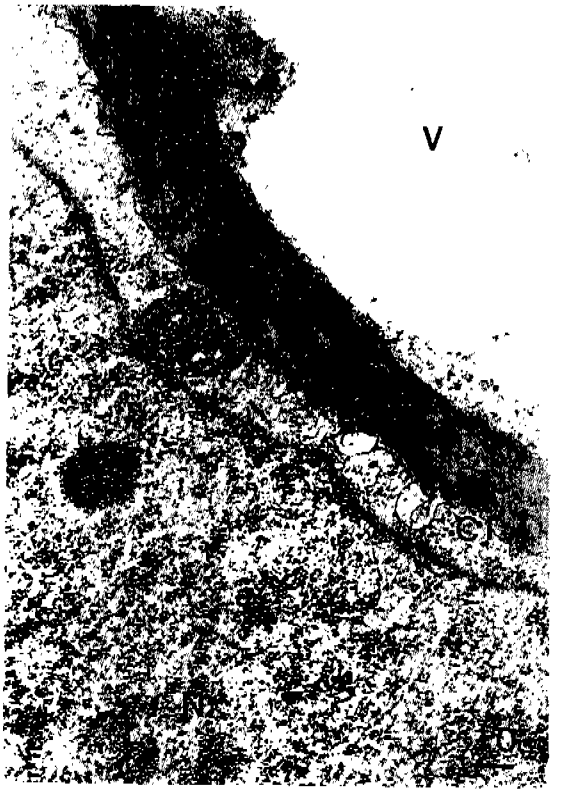
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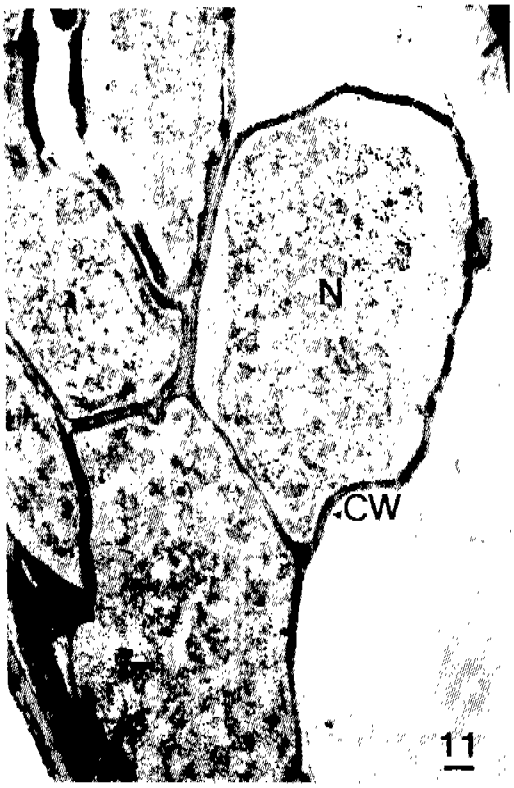
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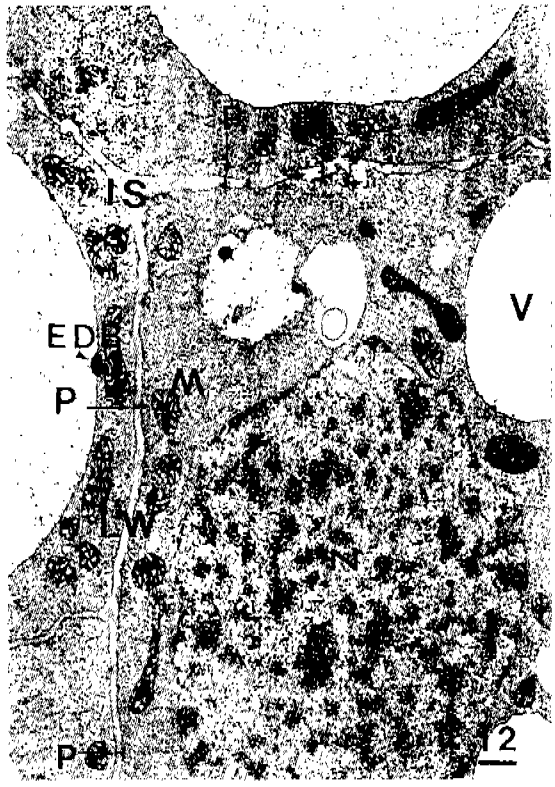
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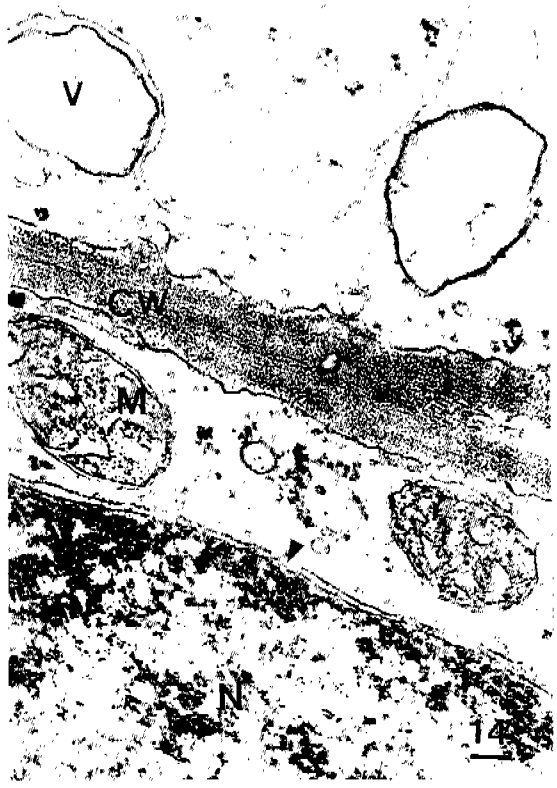
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Fig. 1~5. 1. Photograph of the adventious roots induced from callus of *Nicotiana tabacum* cv. NC 82. cultured for 35 days. RP : Root meristem 2. A light micrograph of the root tip. x 500 3. Several vacuoles containing an electron-dense body. Note a lot of mitochondria are scattered in the cytoplasm near the cell wall. x 4,000 M ; Mitochondria, P ; Plasmodesmata, V ; Vacuole, Nu ; Nucleolus, EDB ; Electron-dense body 4. A large, well developed nucleus and scattered mitochondria with swollen cristae in the cytoplasm. x 6,360 TW ; Transverse, M ; Mitochondria, N ; Nucleus 5. Nucleus within the double membrane is observed. Note the amyloplasts filled with starch grains. Endoplasmic reticulum is small and single filamentous. x 6,360 ER ; Endoplasmic, S ; Starch grain.

Fig. 6~10. 6. Prominent middle lamella and thickened primary cell wall are distinguished characters of callus cells. Note thin cytoplasm with mitochondria and endoplasmic reticulum. x 9,600 ML ; Middle lamella, CI ; Cytoplasmic invagination, M ; Mitochondria, ER ; Endoplasmic reticulum, PC ; Primary cell wall, V ; Vacuole 7. Callus cells with a few small vacuoles and with prominent plastid containing large starch grains. Note electron-dense bands in starch grains. The intercellular spaces are impregnated with dark stained material, and mitochondria are located along the cell wall. x 2,400 S ; Starch grain, IS ; Intercellular space, N ; Nucleus, Nu ; Nucleolus, CY ; Crystalloid, 8. A nucleus located in the vicinity of cell wall. A crystalloid in microbody can be observed. x 3,360 CW ; Cell wall 9. Higher magnification of Fig. 8 showing the microbody surrounded by a membrane. Note a microbody containing a large chrystalline structure. x 63,600 Mb ; Microbody 10. The plasma membrane shows invaginations. x 15,600 PM ;

Fig. 11~14. 11. The cells of the root tip sectioned longitudinally. x 5,200 N ; Nucleus, CW ; Cell wall 12. Hypertrophic nucleus and various shapes of mitochondria in cytoplasm. Note plasmodesmata distribution. x 63,600 P ; Plasmodesmata, IS ; Intercellular space, EDB ; Electron-dense body, V ; Vacuole, M ; Mitochondria, LW ; Longitudinal wall, 13. Numerous spherical mitochondria and thin cell wall without developed intercellular space. x 33,600 14. The nucleus with double-membraned envelope. Note nuclear pore(arrow head). Also, middle lamella and primary cell wall are distinct. x 15,600.

which had round aggregate with electron-dense banded and electon-lucent, non-banded types in the cells of root cap. The cells in the nodules of *Alnus incana* contained starch grains with electron-dense bands distributed near the cell wall (Vikman *et al.*, 1990). Like the report of Phillips and Torrey(1974b), in the cells of the *in vitro* root, the amyloplast filled with starch grains were located adjacent to the cell wall. Nuclei were spherical, but ellipsoidal forms were also observed in the *in vivo* root. Phillips and Torrey (1974a) mentioned that the distinct features of the root meristem cells are dark stained nucleolus and evenly scattered heterochromatin, which coincides with our results.

Callus cells were characterized by following features : the cell size was large and the cells had little cytoplasm. Their nuclei were smaller(as compared with those of the meristem) and situated parietally with one vacuole filling almost the entire volume of the cell. The mitochondria had slightly swollen cristae. Microbody had an ordered rectangular body that was a crystalline form of the enzyme urate oxidase (Căchita and Crăciun, 1990). These ultrastructural features coincide with those of matured cells. Due to the 35 days of culture, callus cells may show the character of maturation to initiation of senescence.

During culture, chloroplasts in the cytoplasm of the leaf cells might have turned into amyloplasts in callus cells ; the whole plastids were filled with starch grains. Maybe this is due to the fact that the growth medium is rich in carbohydrates and during the culture, that the metabolism of the inoculated cells is mostly heterotrophic.

Presence of crystal in microbody and deposits of strong electro-dense inclusions on tonoplast were also reported in *chrysanthemum* callus cells (Cächita and Crăcium, 1980).

There were similar numbers of the plasmodesmata on both walls in the cells either *in vivo* or *in vitro* root, despite a few reports suggested that there were more plasmodesmata on transverse wall than longitudinal wall in the root tip of *Vicia faba* (Griffiths and Audus, 1964) and *Zea mays* (Juniper and Barlow, 1969).

It is very intersesting in similarity of plastid differentiation and mitochondria containing swollen cristae both in the adventitious root and callus cells.

요 약

담배 (NC 82) 잎 절편체의 배양으로부터 형성된 캘러스와 이로부터 유도된 부정근 분열조직의 미세구조를 광학 및 전자현미경을 사용하여 관찰하였다. 근분열조직의 중앙부 세포는 세포 간극이 발달되어 있지 않았고, 2개의 인을 포함하고 있는 구형의 핵이 가끔 관찰되었으며, 약간 팽창된 크리스테를 지닌 구형, 타원형의 미토콘드리아가 세포질 내에 산재되어 있었다. 세포벽에서의 원형질연락사는 수 개가 무리지거나 단독으로 존재하며, 색소체는 비교적 전자밀도가 낮은 수 개의 전분립이 전자밀도가 높은 1~5 개의 띠를 포함하고 있으며, 전분립은 단일막으로 둘러싸여 있었다. 그리고 미소체는 결정체를 지니고 있었다. 캘러스를 구성하고 있는 세포는 두 형태를 지녔는데, 대부분의 세포는 세포질이 하나의 중앙 액포로 채워져 빈약하였으며, 중엽과 일차벽이 발달하였다. 색소체는 부정근 세포의 전분립과 같은 형태를 지녔다. 캘러스와 뿌리의 미세구조의 차이는 전자의 경우, 비후한 세포벽과 큰 액포가 특징적이며, 후자는 얇은 세포벽과 작은 액포들을 가진 점이었다. 전반적으로 캘러스 세포는 성숙한 조직이나 노화된 조직의 특징을 나타내었다.

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