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Ultrastructure and Development of Nonarticulated Laticifers in Seedlings of *Euphorbia maculata* L.

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The ultrastructure of nonarticulated laticifers in the seedlings of *Euphorbia maculata* was studied at various developmental stages. The apical regions of the seedling laticifers growing intrusively contained large nuclei with mainly euchromatin and dense cytoplasm possessing various and many organelles such as rich ribosomes, several small vacuoles, giant mitochondria with dense matrices, rough endoplasmic reticulum, dictyosomes, and proplastids. This result suggested that the apical regions of laticifers were metabolically very active. Laticifers in seedlings at the first-leaf developmental stage did not contain latex particle. In seedlings at second-leaf growth stage, the laticifer cells contained numerous and elongated small vacuoles. These vacuoles appeared to arise by dilation of the endoplasmic reticulum and frequently possessed osmiophilic or electron-dense latex particles. The small vacuoles fused with the large vacuole occupying the central portion of the subapical region of laticifers, and then the latex particles were released into the large central vacuole. The latex particles varied in size and were lightly or darkly stained. Proplastids with a dense matrix and a few osmiophilic plastoglobuli were filled with an elongated starch grain and thus were transformed into amyloplasts. Latex particles were initially produced in the laticifers after seedlings had developed their second young leaves. In seedlings at forth-leaf stage, latex particles with an alveolated rim were found in the laticifers.

Keywords: *Euphorbia maculata*, latex particle, nonarticulated laticifer, ultrastructure

Laticifers are specialized cells with an exudate, a latex or milky sap. The latex consists of various chemical substances such as a complex solution of suspended particles, which are useful economically and biomedicinally (Fahn, 1979; Calvin, 1987). Nonarticulated branched laticifers are characterized by multinucleated cells and their intrusive apical growth (Mahlberg, 1993). This cell type has been studied at the subcellular level in many latex-producing plants. It has commonly been suggested that in several species a large central vacuole develops during the differentiation of laticifers; electron-dense latex particles or osmiophilic globules are then released into the vacuole: *Euphorbia marginata* (Schulze et al., 1967), *Nelumbo nucifera* (Esau and Kosakai, 1975), *Asclepias syriaca* (Wilson and Mahlberg, 1978, 1980), *Ficus carica* (Rachmilevitz and Fahn, 1982), *Euphorbia pulcherrima* (Fineran, 1983). However, cellular components participating in the formation of latex particles differ in the various plant species. Latex particles have been reported to arise in the cytoplasm of *Taraxacum bicorne* (Heinrich, 1967), *A. syriaca* (Wilson and Mahlberg, 1980), and *F. carica* (Rachmilevitz and Fahn, 1982), in Golgi vesicles of *Euphorbia characias* (Marty, 1968), in association with rough endoplasmic reticulum of *Gnetum gnemon* (Bencke and Hermann, 1978), and in small vacuoles of *N. nucifera* (Esau and Kosakai, 1975) and *E. pulcherrima* (Fineran, 1983).

The purpose of this study was to investigate the ultrastructure of nonarticulated laticifers of *Euphorbia maculata* at various developmental stages and to determine the site of the formation of latex particles and the time when the latex particles are produced in this plant.

MATERIALS AND METHODS

Seeds of *E. maculata* L. were soaked on the moist filter paper in petri dishes at in the dark and at 28°C in an incubator. Seedlings with the first young leaves were collected to observe laticifers at the early developmental stage. Greenhouse-grown seedlings which had developed their second to fourth young leaves were collected to characterize the fine structure of laticifers at the subsequent stages.

Whole seedlings were prefixed in 4% glutaraldehyde or 2.5% glutaraldehyde-2% paraformaldehyde

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in 0.1 M sodium cacodylate buffer (pH 6.8) at room temperature for 8-12 h. The shoot apical segments with the young leaves were excised and additionally prefixed for 1-2 h. All materials were postfixed in 1% OsO_4 in 0.1 M sodium cacodylate buffer and dehydrated through an ethyl alcohol/acetone series to anhydrous acetone. Tissue pieces were embedded in Spurr's resin (Spurr, 1969), polymerized at 60°C for 20-24 h, and then sectioned on a Porter-Blum MT-2 ultramicrotome with glass knife. Sections mounted on grids were stained with uranyl acetate and lead citrate. Specimens were examined and photographed with Philips EM 300 transmission electron microscope at 60 kV.

RESULTS

Laticifers were observed in seedlings at various developmental stages of which the first to fourth young leaves had developed. The lengths of the young leaves were in the range of 0.5 mm to 1 mm. In the seedlings, laticifers below the shoot apices were observed.

Using a criterion of cytological features, the laticifers below the shoot apices could be distinguished into two regions: i) apical regions growing intrusively between neighboring parenchymatous cells, and ii) subapical regions located at the first internode in which large central vacuoles were developed. In this study the fine structure of laticifers in second-leaf stage seedlings will mainly be described.

Apical Regions

The diameter of the apical regions of laticifers was narrower compared with that of remaining subapical regions. The apical regions grew into intercellular spaces of adjacent parenchymatous cells. Nuclei were large and contained mainly euchromatin at the apical regions. Dense cytoplasm contained rich ribosomes, several small vacuoles, various cell organelles such as mitochondria, rough endoplasmic reticulum (r-ER), dictyosomes, and proplastids (Figs. 1 and 2). Giant mitochondria with dense matrices and well-developed cristae were frequently observed. Elongated proplastids contained dense matrices, and a few osmiophilic plastoglobuli were found, while no thylakoid membranes and starch grains were observed. However, chloroplasts in adjacent parenchymatous cells had well-developed granal thylakoids. This feature of the plastids allowed us to easily detect the laticifers growing within neighboring parenchymatous cells. No plasmodesmatal connections between the laticifer and parenchyma cells were found. Cell walls of laticifers were somewhat thicker than those of adjacent parenchymatous cells. Such cytological features were also similarly observed at the apical regions growing intrusively in the seedlings at first- to fourth-leaf developmental stages.

Cisternae of the ER were partially dilated and formed elongated or tubular small vacuoles (Figs. 3 and 4). The elongated small vacuoles contained electron-dense particles or osmiophilic globules (Figs. 1, 4, and 5), and their vacuolar membranes were fused to each other (Figs. 2 and 3). The vacuolation of ER cisternae and the fusion of the vacuolar membranes were also visible at the subapical regions.

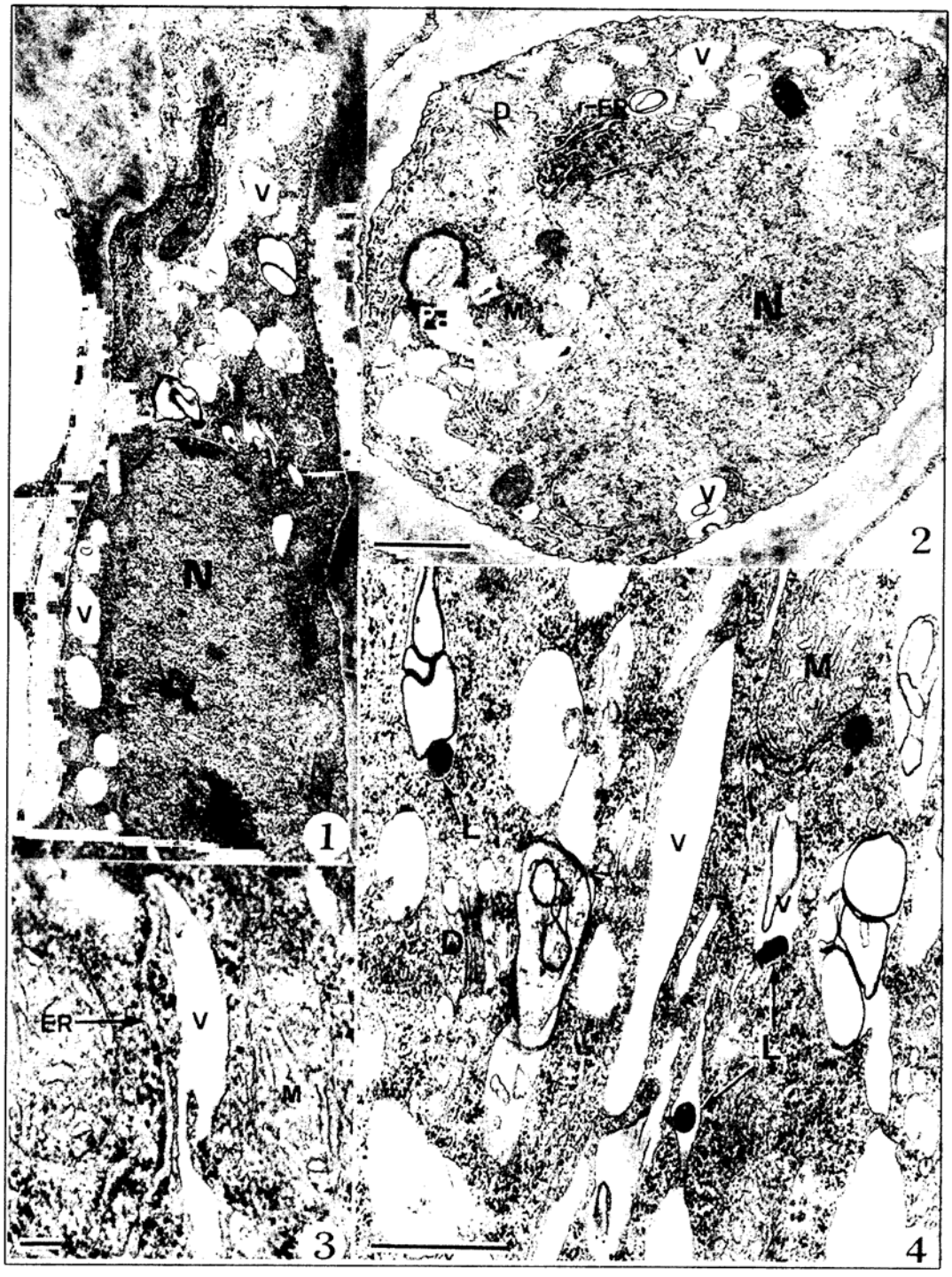
Subapical Regions

A large vacuole occupied the central portion of the subapical region of laticifers, whereas several small and elongated vacuoles were located in a thin layer of peripheral cytoplasm. In the laticifers of first-leaf stage seedlings, latex particles were not found in the central vacuole and in the peripheral cytoplasm of laticifers, small vacuoles were fused with a central one, and no plastids had starch grains and thylakoid membranes (Fig. 6).

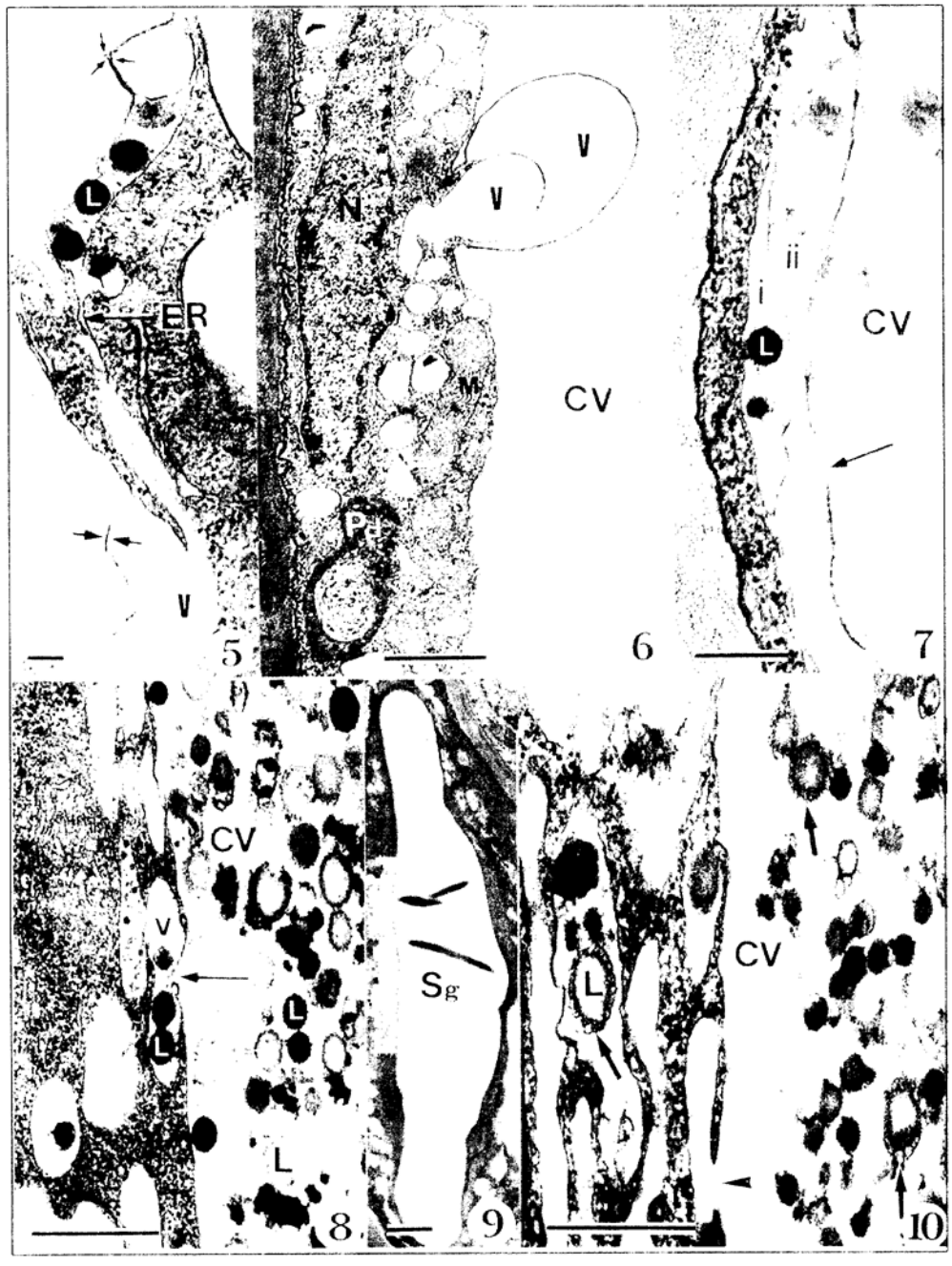
In second-leaf stage seedlings, the vacuoles containing latex particles gradually fused with a large central vacuole (Fig. 7), and the particles were released into the central vacuole (Fig. 8). The size of the latex particles varied and the particles were densely or lightly stained. At this stage, plastids had no thylakoid membrane, but were filled with an elongated or rod-shaped starch grain, and eventually transformed into amyloplasts (Fig. 9). In laticifers below the shoot apices of fourth-leaf stage seedlings, frothy lightly-stained latex particles were frequently observed in elongated vacuoles and in the large central vacuole (Fig. 10).

DISCUSSION

One of the most striking features of the nonarticulated laticifers is the branching and intrusive growth at the tip regions between adjacent cells (Mahlberg, 1993). In these regions, the dense cytoplasm contained large nuclei with euchromatin and various organelles, including ribosomes, r-ER, mitochondria, dictyosomes, and proplastids. Such cytological features indicated that the metabolism at the apical



Figures 1-4. Electron micrographs of the apical regions of laticifers below the shoot apices of *I. maculata* seedlings with the second young leaves. **1.** An apical region of laticifer sectioned longitudinally has a large elongated nucleus (N) and a dense cytoplasm including rich ribosomes, several small vacuoles (v), proplastids (Pp) with a dense matrix and a few osmiophilic plastoglobuli. Bar=1 µm. **2.** A transverse view of the apical region of a laticifer shows a large nucleus (N) with euchromatins and various cell organelles such as mitochondria (M), rough endoplasmic reticulum (r ER), dictyosome (D), proplastids (Pp). Bar=1 µm. **3.** Cisterna of ER is partially dilated and forms an elongated small vacuole (v). M, mitochondria. Bar=0.1 µm. **4.** Several small and elongated vacuoles (v) containing darkly stained latex particles (L) and membranous structures. D, dictyosomes; M, mitochondria. Bar=0.5 µm.



Figures 5-10. Electron micrographs of the apical (Fig. 5) and subapical regions (Figs. 6-10) of laticifers below the shoot apices of *E. maculata* seedlings with first (Fig. 6), second (Figs. 5, 7-9), and fourth (Fig. 10) young leaves. **5.** An elongated vacuole containing latex particles (L) is connected with another one (v) by ER cisterna. Small vacuoles are fusing each other (arrows). Bar=0.1 μm. **6.** Transversely sectioned laticifer. No latex particles are visible in the numerous small vacuoles (v) at the peripheral cytoplasm and in a central large one (CV). Two small vacuoles (v) are incorporating into a CV. Proplastid (Pp) has a dense matrix and a few osmiophilic plastoglobuli. N, nucleus; M, mitochondria. Bar=0.1 μm. **7.** Transversely sectioned laticifer. A small vacuole (i) containing latex particles (L) is fusing with another one (ii). They are also fusing with a central large vacuole (CV). The vacuolar membrane of the small vacuole (ii) appears to be partially degraded (arrow). Bar=0.5 μm. **8.** Longitudinally sectioned laticifer. A large central vacuole (CV) contains numerous darkly or lightly stained latex particles (L). Small vacuoles (v) possessing latex particles (L) in the peripheral cytoplasm are fusing (arrow) with a CV. Bar=0.5 μm. **9.** A plastid of laticifer has a large and elongated starch grain (Sg), but no thylakoid membrane. Bar=1 μm. **10.** Another type of latex particles (L), with an alveolated rim (arrows) in the elongated vacuoles (v) at the peripheral cytoplasm and in the central large vacuole (CV). Arrowhead indicates where a small vacuole is fused with a large central one. Bar=0.5 μm.

region was in a highly active state, which would contribute to the intrusive growth of the laticifer tips. At the apical regions, enzymes like pectinase may be synthesized and secreted, which may solubilize pectic substance of the middle lamella of adjacent cell walls and facilitate the apical intrusive growth (Wilson et al., 1976).

The rubber component of latex is usually visualized as the osmiophilic globules in electron microscopy (Wilson and Mahlberg, 1980). These electron-dense particles have been identified as latex particles in the laticifers of many other latex-producing plants studied. It has been found that various cellular components are associated with the formation of latex particles in different species (Datta and Iqbal, 1994). In *E. maculata*, dilated ER cisternae were transformed into elongated small vacuoles, in which electron-dense latex particles were formed. This feature was very similar to those of the laticifers of *N. nucifera* (Esau and Kosakai, 1975), *A. syriaca* (Wilson and Mahlberg, 1978) and *E. pulcherrima* (Fineran, 1983). On the other hand, the latex particles of laticifers may originate in the cytoplasm in *T. bicorne* (Heinrich, 1967), *A. syriaca* (Wilson and Mahlberg, 1980), and *F. carica* (Rachmilevitz and Fahn, 1982), in Golgi vesicles in *E. characias* (Marty, 1968), in association with rough endoplasmic reticulum in *G. gnemon* (Bencke and Herrmann, 1978), and in small vacuoles in *N. nucifera* (Esau and Kosakai, 1975) and *E. pulcherrima* (Fineran, 1983).

An alveolated type of latex particles observed in the laticifers of forth-leaf stage seedlings was not reported in the other latex-bearing plant. In several plant species studied, electron-dense latex particles are usually round and variably sized. In laticifers of *A. syriaca*, latex particles are coated with electron-dense fibrilar-granular material (Wilson and Mahlberg, 1980).

In *E. pulcherrima*, the electron-dense matrix of the latex particles may originate from the vacuolar sap (Fineran, 1983). However, we could not detect the origin of the latex particles matrix in *E. maculata*. The origin and chemical nature of latex particles in *E. maculata* would need to be addressed in subsequent work.

No plastids with thylakoid membrane and starch grain were found at the apical regions of the laticifers in all seedlings observed. Plastids existed as proplastids and were visible at the subapical regions of the laticifers in seedlings with the first young leaves. However, the proplastids were filled with an elongated starch grain and so were transformed into amyloplasts during the second-leaf growth stage. Mahlberg (1975) has suggested that the elongated starch grains in laticifers are derived from the round or oblong ones in parenchymaous cells. It is thought that a discoid type of starch grain is phylogenetically considered to be the most specialized and an osteoid one is intermediate. In *Calotropis*, the parenchyma cells have round or oblong starch grains which are aggregated to form an osteoid type in laticifers (Datta and De, 1986). Starch accumulated in laticifers does not utilize during plant growth (Biesboer and Mahlberg, 1978). It is conceivable that laticifer starch grains might carry out any function like plant protection. Considering that osteoid type of starch grain can aggregate with itself easier than round or oblong type, the osteoid type may be more beneficial in plant protection. It could be interpreted that more specialized type of starch grain like osteoid or discoid type may more effective in occluding laticiferous tubes and in preventing the bleeding of latex contents, similar to as the formation of blood clotting in animals when tissues are cut.

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