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Microtubular configurations during the cellularization of coenocytic endosperm in *Ranunculus sceleratus* L.

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Summary. Endosperm cellularization in *Ranunculus sceleratus* was studied in terms of the initiation of cell-wall formation in the coenocytic endosperm. The first endosperm cell walls were in an anticlinal position relative to the cell wall of the embryo sac and originated from the cell plates and not from wall ingrowths from the embryo-sac wall itself. Alveolar endosperm was formed 3 days after pollination. Microtubules were associated with the freely growing wall ends of the anticlinal walls and were observed in various orientations that generally ranged from angles of 45° to 90° to the plane of the wall. They were absent in the regions where vesicles had already fused. These microtubules may function in maintaining the growth and the direction of growth of the anticlinal wall until cellularization is completed. At the site where three neighbouring alveoli share their freely growing wall ends, remarkable configurations of microtubules were observed: in each alveolus, microtubules ran predominantly parallel to the bisector of the angle formed by the common walls. These microtubules may form a physically stable framework and maintain the direction of growth of the wall edges. It is concluded that the growing edge of the anticlinal endosperm wall and its associated microtubules are a special continuum of the original phragmoplast that gave rise to the anticlinal wall.

Key words: Cell wall – Cytoskeleton – Endosperm cellularization – Microtubules – *Ranunculus sceleratus*

Introduction

Angiosperms with the nuclear type of endosperm development initially exhibit coenocytic endosperm. Endosperm cell-wall formation begins when the endosperm has formed one layer of nuclei lying near the cell wall of the central cell.

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The process of wall formation has been studied by electron microscopy in various species. In *Triticum aestivum* it has been reported that cell walls originate, at least in part, from cell-wall protrusions (Mares et al. 1977; Morrison et al. 1978). This has also been found in *Helianthus annuus* (Newcomb 1973; Yan et al. 1991), *Arabidopsis thaliana* (Mansfield and Briarty 1990) and *Stellaria media* (Newcomb and Fowke 1973). However, Fineran et al. (1982) and Van Lammeren (1988) concluded that endosperm cell walls in *T. aestivum* originate from cell plates. The literature is also contradictory concerning the structure of the growing wall ends. Three types have been described: (1) wall edges without fusing vesicles and microtubules (MTs), (2) wall edges with fusing vesicles but without MTs and (3) wall edges with both fusing vesicles and MTs (For a review see Fineran et al. 1982).

The present paper presents data on the origin of the first endosperm cell walls in *Ranunculus sceleratus* and on the configuration and function of the MTs associated with the growing wall ends during the initial phase of endosperm cellularization.

Materials and methods

Plants of *Ranunculus sceleratus* L. were grown under greenhouse conditions. Developing ovaries and ovules were excised at various days after anthesis (DAA). For both electron microscopy and conventional light microscopy, samples were fixed at room temperature for 3–12 h in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate-buffered saline (PBS: 135 m*M* NaCl, 2.7 m*M* KCl, 1.5 m*M* KH_2PO_4, 8 m*M* Na_2HPO_4, pH 7.2). After rinsing in the same buffer, samples were post-fixed for 12 h at 4° C in 1–2% osmium tetroxide in PBS, then rinsed in PBS and dehydrated in a graded ethanol series. The long fixation was necessary to preserve ultrastructure and microtubules. Samples were embedded in low-viscosity resin (Spurr 1969). For light microscopy semithin median sections were stained with Toluidine Blue O. For electron microscopy, ultrathin sections were stained with uranyl acetate and lead citrate and analysed using a JEM-1200 EXII transmission electron microscope operating at 80 kV.

For the immunocytochemical staining of MTs, samples were fixed in 4% paraformaldehyde in microtubule stabilizing buffer (MSB: 1 m*M* EGTA; 4% PEG; 100 m*M* PIPES, pH 6.9) containing 0.05% Triton X-100 (w/v) for 3 h at room temperature. After rinsing in PBS and infusion with 1 *M* sucrose for 12 h followed by an infusion with 2.3 *M* sucrose in PBS for 24 h, samples were

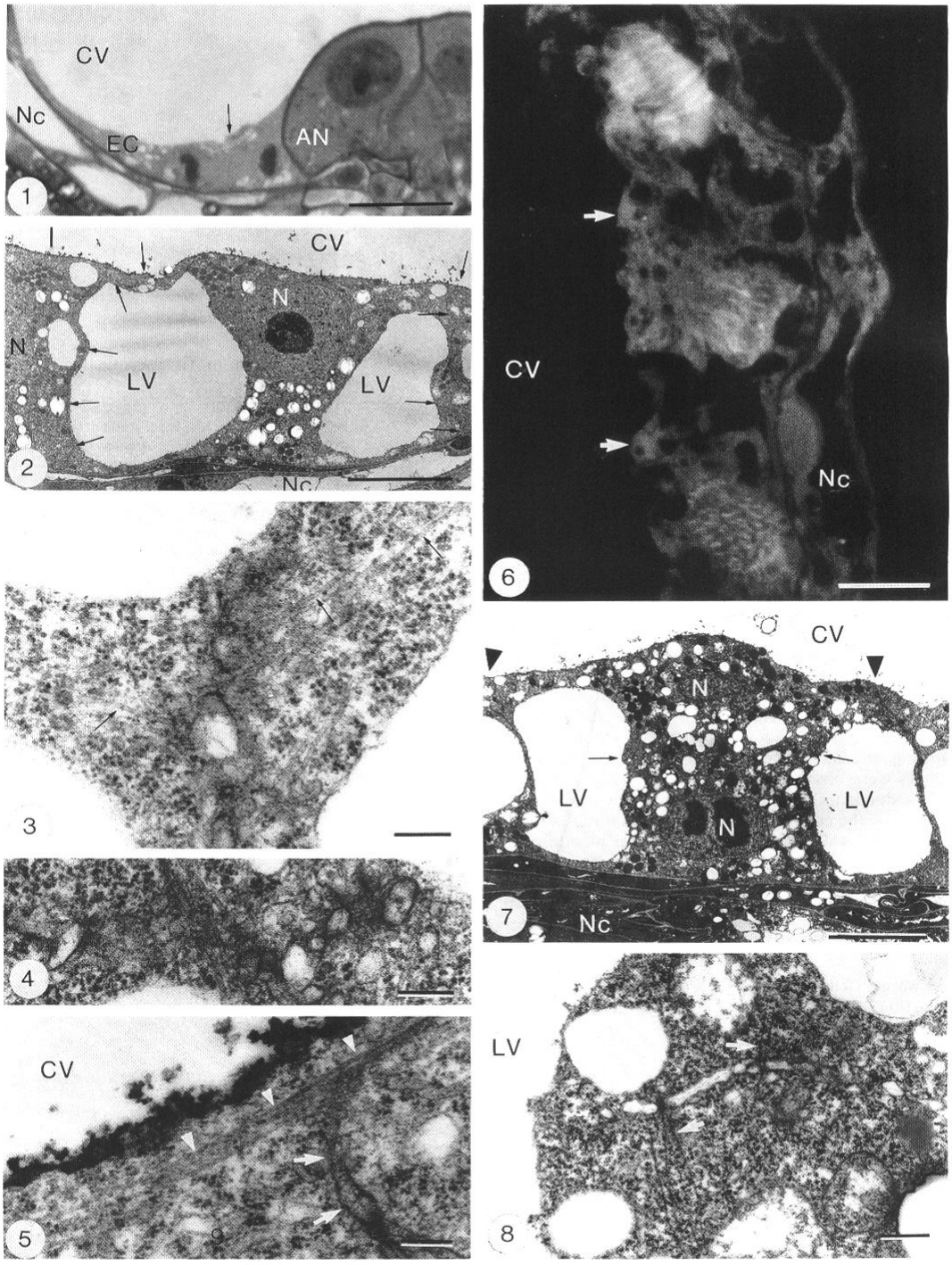


Fig. 1–8

cryofixed in liquid nitrogen sludge. Semi-thin cryosections were prepared with a Reichert Jung FC 4D ultra-cryomicrotome and affixed to poly-L-lysine-coated slides (poly-L-lysine, Sigma, St Louis, Mo., USA; 0.1% (w/v) in PBS, pH 7.2). The sections were incubated with rabbit polyclonal antitubulin (R229, see Van Lammeren et al. 1985) diluted in PBS (1:40), labelled secondly with GaR-FITC (Nordic-Tilburg-Holland) and observed with a Nikon Labophot epifluorescence microscope (for details see Van Lammeren 1988).

The pattern of endosperm development was investigated in median sections of the developing seeds, primarily in the ventral and dorsal regions of the endosperm.

Results

Formation of alveolar endosperm

Three days after anthesis a layer of cytoplasm with free endosperm nuclei has formed in the embryo sac. We observed a monolayer of endosperm nuclei in semi-thin, cryosections of an ovule; arrays of MTs radiated from each nuclear envelope. The first nuclear division, which coincided with cell-plate formation, occurred in the chalazal part of the endosperm (Fig. 1). The cell plate was aligned perpendicular to the wall of the embryo sac. On one side the cell plate extended towards and fused with the wall of the embryo sac; on the opposite side it grew towards the central vacuole of the embryo sac and maintained a freely growing end. Thus, a cell wall was formed that was in an anticlinal position relative to the embryo sac wall. More anticlinal walls were formed after successive divisions. These cell walls created an alveolar coenocyte. Each alveolus contained a nucleus but had no cell wall on the side facing the central vacuole (Fig. 2). In general, the nucleus was located near the tonoplast of the central vacuole. Alveolar vacuoles were found between the nucleus and the anticlinal walls.

Structure of the anticlinal wall edge

Anticlinal wall edges consisted of numerous small membranous vesicles (Fig. 3). Extensive arrays of MTs were found associated with the wall edges that could only be visualized electron microscopically by prolonged fixation. Generally, the growing wall edge-associated MTs (GWE-MTs) extended more than 5 µm into the surrounding cytoplasm on both sides of the wall plane. Numerous GWE-MTs stopped at the growing wall end in darkly stained areas on the surfaces of membranous vesicles (Fig. 3), some penetrated the wall edge (Fig. 4) and some were observed between the wall edge and the tonoplast of the central vacuole (Fig. 5). The orientation of the GWE-MTs varied. Most GWE-MTs were oriented at a 45°–90° angle to the plane of the wall edge (Figs. 3–5); however, some were found parallel to the new wall. GWE-MTs were absent in the region where vesicles had fused and had formed smooth plasmalemma (Fig. 5).

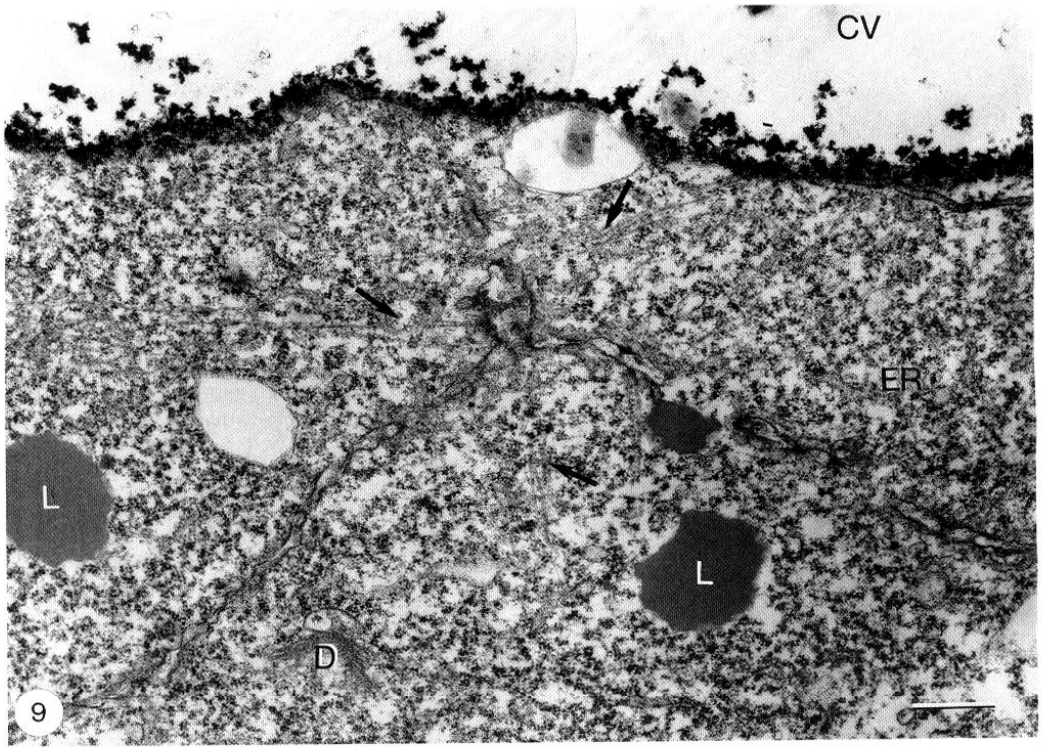
Cellularization of alveolar endosperm

By 4 days after anthesis the nuclei in the alveoli had divided (Fig. 6). The equatorial plate was parallel to the embryo-sac wall, and cytokinesis had given rise to a layer of uninucleate cells adjacent to the embryo-sac wall and a layer of alveoli bordering the central vacuole (Fig. 7). The newly formed periclinal cell plate between two daughter nuclei could not connect with the anticlinal walls immediately (Fig. 7) but had to extend and push the tonoplasts of the lateral vacuoles towards the anticlinal walls. The growing wall edges extended by adding vesicles to their very ends (Fig. 8). Microtubules were associated with the growing wall edge, and the orientation of the MTs was mainly at an angle between 45° and 90° to the cell wall, while some MTs penetrated the growing edge. In the area where the new wall had been established, perpendicularly oriented MTs were not observed. The following division in the alveoli resulted in a second layer of endosperm cells, whereas the alveoli remained. This cellularization continued until alveoli met in the centre of the embryo sac.

Microtubular configurations in the contact region of three neighbouring alveoli

Anticlinal wall formation resulted in a regular arrangement of alveoli with a honeycomb appearance. In trans-

Figs. 1–8. *AN* Antipodals, *CV* central vacuole, *EC* endosperm cytoplasm, *LV* lateral vacuole, *N* nucleus, *Nc* nucellus. **Fig. 1.** Light micrograph of nuclear endosperm and antipodals in the chalazal part of the embryo sac of *R. sceleratus*. The endosperm cytoplasm and nuclei lie between the sac wall and the central vacuole. Note the first karyokinesis, which coincides with the formation of the cell plate (*arrow*). *Bar*: 20 µm. **Fig. 2.** Electron micrograph of endosperm of *R. sceleratus* at the alveolar stage of development. Note the anticlinal walls (*arrows*), which separate the nuclei and form the alveolus. *Bar*: 10 µm. **Fig. 3.** Electron micrograph of the growing edges of an anticlinal wall in the alveolar endosperm. Microtubules run along both sides of the growing wall edge (*arrows*) and connect with the darkly stained surfaces of vesicles. *Bar*: 200 nm. **Fig. 4.** Electron micrograph of the growing edge of an anticlinal wall in the alveolar endosperm. Some GWE-MTs penetrate the growing edges of an anticlinal wall. *Bar*: 200 nm. **Fig. 5.** Electron micrograph of the growing edge of an anticlinal wall at the alveolar endosperm stage showing a bundle of MTs (*arrowheads*) between the growing wall edge (*arrows*) and the tonoplast of the central vacuole. *Bar*: 200 nm. **Fig. 6.** Light micrograph of immunochemically stained MTs in a semithin cryosection of the endosperm at a late alveolar stage (5 DAA) and just before cellularization by periclinal cell-plate formation. Three alveoli with mitotic spindles are shown. The areas of the growing wall edges of the anticlinal walls are indicated by *arrows*. *Bar*: 10 µm. **Fig. 7.** Electron micrograph showing the early alveolar-cellular endosperm stage. Two nuclei are present in the alveolus. Cell-plate formation (*arrows*) continues underneath the tonoplasts of the lateral vacuoles and will result in a periclinal cell wall forming an endosperm cell and an aveolus. The freely growing wall ends of the alveolus are indicated by *arrowheads*. *Bar*: 5 µm. **Fig. 8.** Detailled view of the edge of the periclinal cell plate as shown in another section in Fig. 7. Note that the MTs are associated with the edge of the cell plate (*arrows*). *Bar*: 500 nm.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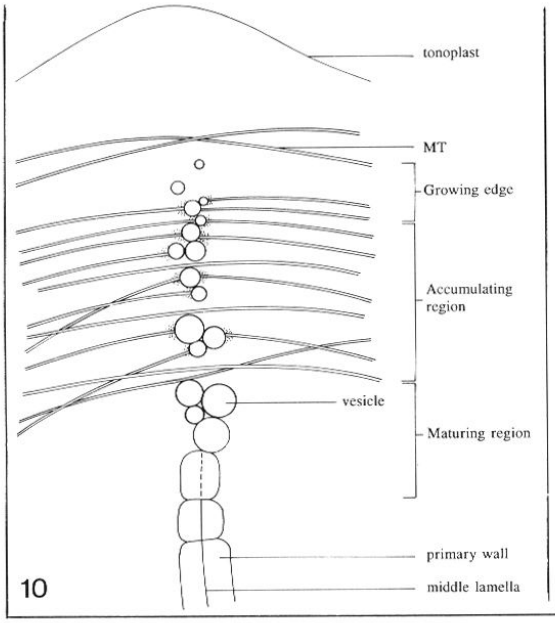
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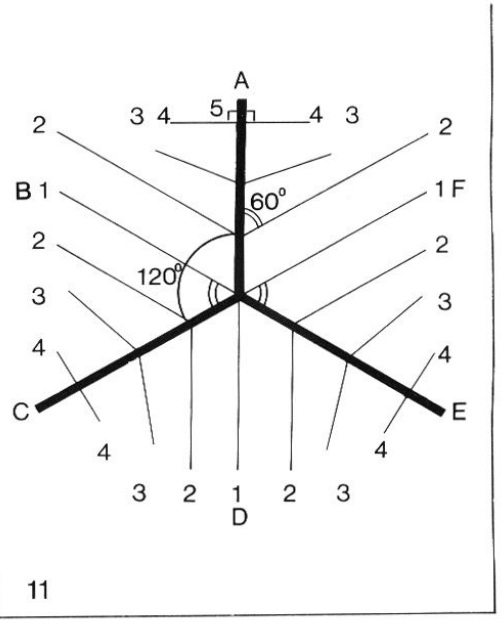
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