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Cell-wall texture in shoot apex cells

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SUMMARY

The common opinion that cellulose microfibril deposition in cell walls of meristematic cells is random will have to be revised. In tunica, corpus and rib meristem cells of *Petunia hybrida* and *Vinca major* microfibrils are deposited in parallel arrays. The cell walls of corpus cells consist of lamellae. Each lamella has a different microfibril orientation. As a consequence, the random distribution of cortical microtubules found in corpus cells (Sakaguchi *et al.* 1988) bear no direct relation to the parallel deposition of cellulose microfibrils in the walls of these cells.

Key-words: cellulose microfibrils, cell-wall texture, shoot apex.

INTRODUCTION

In the vegetative shoot apex of angiosperms, three major regions can be distinguished with specific patterns of cell arrangement (Gifford & Corson 1971):

(a) the tunica region at the tip of the apex, containing one to five layers with division walls perpendicular to the surface (anticlinal);

(b) the corpus region underneath the tunica, with division walls oriented at random;

(c) the rib meristem, i.e. the region below the corpus which extends to the pith of the stem, with transversal division walls, resulting in longitudinal cell files.

After division of a meristem cell, a new cell wall is formed around each daughter cell. As the sister cells elongate during the growth following division, each newly formed cell comprises at least two cell walls when the mother cell-wall is not damaged too much from tearing during growth (Esau 1977, Wolters-Arts & Sassen 1991). Tunica cells and rib meristem cells divide in a direction perpendicular to the growth direction of the sister cells. Cellulose microfibrils are deposited in arrays parallel to these cell walls and perpendicular to the growth direction (Wardrop *et al.* 1979). Corpus cells divide and grow in various directions, while the microfibrils are randomly deposited (Frey-Wyssling 1959).

The aim of this study was to investigate the cell wall texture of tunica, corpus and rib meristem cells of *Petunia hybrida* electron-microscopically. As the results of Sakaguchi *et al.* (1988) on cellulose microfibril deposition in shoot apex cells of *Vinca major*, obtained by use of polarized light microscopy, are disputable, the shoot apex of *Vinca major* was also studied electron-microscopically. It is well known that studies using polarized light give the overall cellulose microfibril orientation of a cell wall and do not give any information on the orientation of the last deposited microfibrils (Preston 1974, Green 1980, Hogetsu 1986).

Dedicated to Professor H. F. Linskens, Nijmegen, on the occasion of his 70th birthday in 1991.

MATERIALS AND METHODS

Plant material

Vegetative shoot apices were collected from *Petunia hybrida* L. plants, grown under greenhouse conditions. Shoot apices from *Vinca major* plants, grown in the garden, were collected in July and August. Wall synthesis was stopped by fixation in 2% glutaraldehyde in phosphate buffer pH 7·6, for 1 h.

Light microscopy

Fixed shoot apices were dehydrated and embedded in epoxy resin (Spurr 1969). Sections of 2 μm thick were cut and stained with toluidine blue.

Electron microscopy

To visualize the cellulose microfibrils on the inner surface of the cell walls, fixed shoot apices were embedded in polyethylene glycol (Hawes *et al.* 1983, Wilms 1989). Median longitudinal sections, 5 μm thick, were clamped in nickel oyster grids coated with a formvar film. The sections were extracted with hydrogen peroxide 30% and acetic acid 96% 1:1 for 1 h at 90°C. After rinsing with water, the grids were transferred to loops covered with a formvar film, then air-dried and Pt-shadowed at an angle of 45°. The cell-wall pattern was not disturbed by this treatment. Preparations were examined with a Jeol EM CX 100 II.

RESULTS

The vegetative shoot apex of *Petunia hybrida* consists of a two-layered tunica, a corpus and a rib meristem (Fig. 1a), like the shoot apex of *Vinca major* (Sakaguchi *et al.* 1988). Figure 1b shows a part of an apex of *Petunia* after treatment with hydrogen peroxide and acetic acid, with two rows of tunica cells (T) and some corpus cells (C). The darker contours are parts of the cell walls which have fallen down during the treatment, while the lighter parts show either cell lumina or the inner surfaces of cell walls in surface view. As the cell-wall pattern was not disturbed, the walls may be composed of at least two superimposed cell walls.

In tunica cells, the cellulose microfibrils were deposited in arrays parallel to the anticlinical cell walls and perpendicular to the growth direction (Fig. 1c). The direction in which microfibrils were deposited in rib-meristem cell-walls was also in arrays parallel to the newly formed cell walls and perpendicular to the growth direction (Fig. 1d). Primary pit-fields of different sizes were present and microfibrils deviated around and crossed the primary pit-fields. In corpus cells, the microfibrils were deposited parallel to one another in lamellae. In contrast to the tunica and rib-meristem cells, the direction of the microfibrils differed in each cell with respect to the longitudinal axis of the shoot apex. The thickness of the walls in relation to the density of the microfibrils was variable. Relatively thin walls showed at least two predominant microfibril orientations (Fig. 1e). The last deposited microfibrils were often situated in bundles which were oriented in parallel to each other and were occasionally interwoven with microfibrils in the underlying lamellae. These cell walls showed small primary pit-fields (not shown). In cells with a relatively thicker wall (not shown), only one predominantly parallel microfibril orientation was present. Pit-fields were larger in thicker walls.

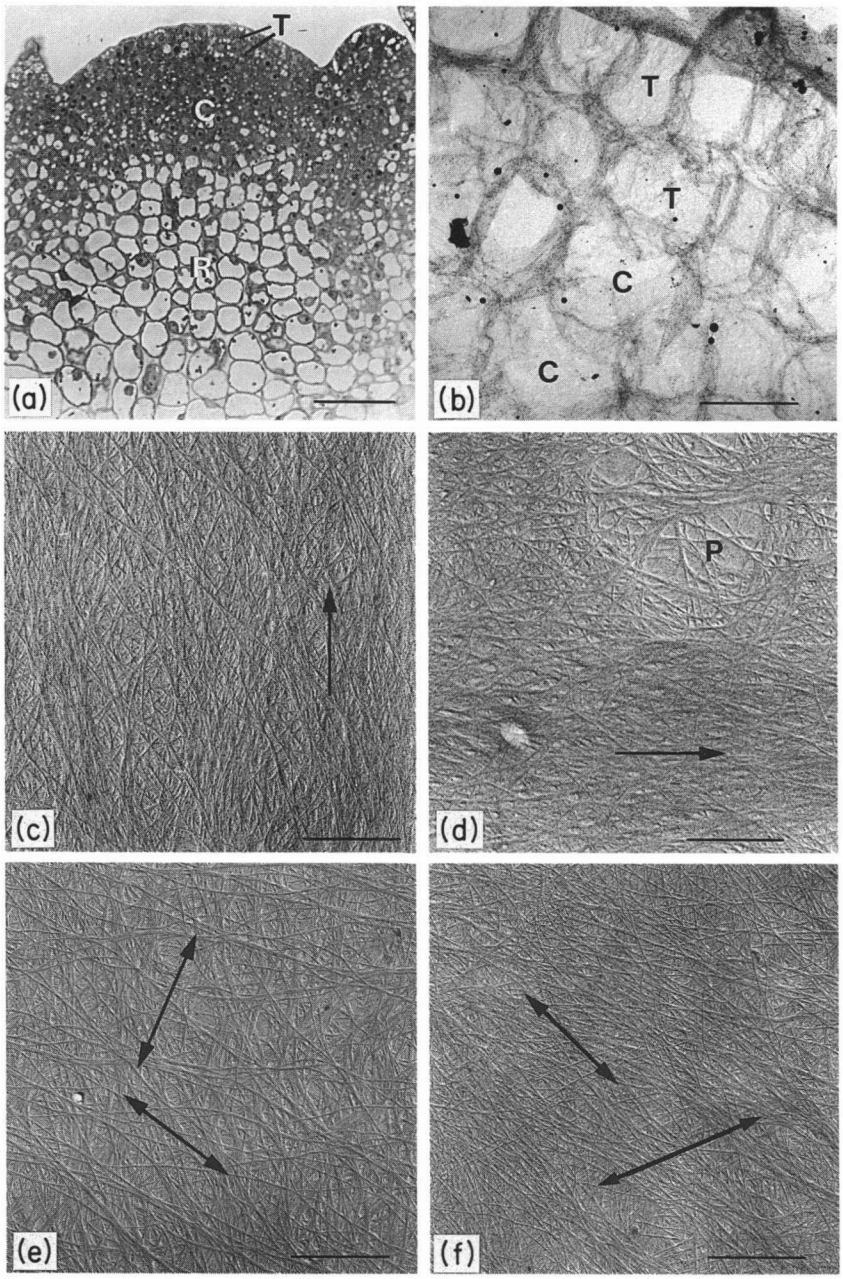

Fig. 1. (a) Median longitudinal section of *Petunia hybrida* shoot apex showing the layered tunica, the corpus and the rib meristem (× 200). Bar = 50 μm. (b) Part of a median longitudinal section of *Petunia hybrida* shoot apex, showing tunica and corpus cells, after treatment with hydrogen peroxide and acetic acid. A few cells of the tunica and corpus are visible (× 1500). Bar = 10 μm. (c) Cell-wall texture of a tunica cell of *Petunia hybrida* with microfibrils parallel to the division wall. The arrow represents the direction of the newly formed cell wall, i.e. perpendicular to the direction of growth. (× 30 000). Bar = 0·5 μm. (d) Cell-wall texture of a rib meristem cell of *Petunia hybrida* with microfibrils parallel to the division wall. The arrow represents the direction of the newly formed cell wall, i.e. perpendicular to the direction of growth (× 30 000). Bar = 0·5 μm. (e) Cell-wall texture of a corpus cell of *Petunia hybrida* with arrays of parallel microfibrils. The arrows indicate the direction of microfibril orientation in two superimposed cell wall lamellae (× 30 000). Bar = 0·5 μm. (f) Cell-wall texture of a corpus cell of *Vinca major*. The arrows indicate the direction of microfibril orientation in two superimposed cell wall lamellae (× 30 000). Bar = 0·5 μm. Abbreviations: C = corpus; P = primary pit-field; R = rib meristem; T = tunica.

In *Vinca major* vegetative shoot apices, the cell-wall textures of the tunica and rib meristem were more or less the same as those of *Petunia*. In both cases the direction of the last deposited microfibrils were perpendicular to the growth direction (not shown). Figure 1f shows the texture of a corpus cell wall in *Vinca*. The last deposited microfibrils were oriented parallel to each other. The inner walls showed 1–2 predominant orientations of microfibrils.

DISCUSSION

As far as the tunica and rib-meristem cells are concerned, we did find the same results as Sakaguchi and co-workers (1988) found in using polarized light microscopy. In both cell types, the microfibrils are deposited in arrays parallel to the division walls and perpendicular to the growth direction. Moreover, this direction is the same in neighbouring cell walls. In such cases, polarized light microscopy visualizes well the overall parallel arrangement of microfibrils.

For corpus cells our findings differed from those of Sakaguchi and co-workers (1988) who used polarized light microscopy. Photographs clearly showed predominant microfibril orientations in different lamellae of the cell wall. Moreover, in the meristem, the cell walls may consist of two or more cell walls dependent on the number of cell divisions (Esau 1977) and even underlying walls of neighbouring cells. The last deposited microfibril orientation differed in each corpus cell; therefore, in polarized light, a random texture of corpus cell-walls was found. Also, for root hair cell-walls which show a helicoidal texture consisting of a large number of superimposed lamellae with a progressive change in microfibril orientation in subsequent lamellae, different data from polarized light microscopy and electron microscopical studies (Sassen *et al.* 1981) were found. The common idea that cells from a randomly dividing tissue have microfibrils deposited at random is only based on investigation with the polarizing microscope (Frey-Wyssling 1959). In fact, the microfibrils are laid down more or less parallel to each other and most probably parallel to the last division wall. Sakaguchi and co-workers (1988) presented the overall orientation of microfibrils in corpus cell-walls, by use of polarized light microscopy. Consequently, the randomly distributed cortical microtubules in corpus cells have no direct relation with the parallel microfibrils in the cell walls of the same cells. In tunica and rib-meristem cells, however, an arrangement of microfibrils and microtubules parallel to one another may exist. The common opinion that cortical microtubules orient newly deposited microfibrils is not found in this study nor in an increasing number of other studies (see review: Emons *et al.* 1991).

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