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Orientation of Wall Microfibrils in *Avena* Coleoptiles and Mesocotyls and in *Pisum* Epicotyls

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Microfibrils (MFs) on the inner surface of the walls of *Avena* coleoptile and mesocotyl cells and of *Pisum* epicotyl cells were examined by a replica method. In the elongating epidermis of these three organs, cells having MFs that were transverse, oblique or longitudinal to the elongation axis were intermingled. In the elongating parenchymal tissues, all cells deposited MFs transversely. In non-elongating cells of *Avena* coleoptiles and *Pisum* epicotyls, the orientation of MFs on the inner wall surface of both epidermal and parenchymal cells was more longitudinal than in elongating cells. These observations on the orientations of MFs are compatible with those our previously reported observations on the orientations of microtubules (MT) (Iwata and Hogetsu 1988). Disruption of MTs of *Avena* coleoptiles by treatment with amiprophosmethyl caused changes in the orientation of deposition of MFs. These results support the idea that MFs are usually co-aligned with MTs in organ cells and that the orientation of MFs is controlled by MTs.

The averaged direction of MFs, visualized under polarized light, showed a clear difference between the epidermal and inner-tissue cell walls in the elongating regions of the three organs. In almost all elongating and non-elongating epidermal cells, the averaged direction of MFs was longitudinal, while it was transverse in all inner-tissue cells.

Key words: Amiprophosmethyl (APM) — *Avena* coleoptile — *Avena* mesocotyl — Microfibril — Organ elongation — *Pisum* epicotyl.

Since Ledbetter and Porter (1963) observed the parallel alignment of MTs and MFs in roots of several species of plants, many electron microscopic observations on the parallel co-alignment of MTs and MFs in tissue cells of higher plants have been reported (Robinson and Quader 1982, for review). Mainly as a result of these observations, it has been postulated that MTs are involved in controlling the orientation of MFs (Green 1980, Gunning and Hardham 1982). Recently, immunofluorescence techniques have been applied to detection of the arrangement of MTs in extensive regions of the tissues of several organs, and it has been found that MTs are arranged specifically depending on the position and age of the cells (Hogetsu and Oshima 1986, Hogetsu 1986, Sakaguchi et al. 1988). In these cases, the orientation of MFs was found to be parallel to that of MTs. However, only a few studies that examined the parallelism of orientations of MTs and MFs over wide regions in plant organs do not allow us to generalize such parallelism in plant organs. More systems need to be examined for a complete analysis of the parallelism between the orientations of MFs and MTs.

In our preceding paper (Iwata and Hogetsu 1988), we reported that, in *Avena* coleoptiles and mesocotyls and in *Pisum* epicotyls, elongating inner-tissue cells have transverse MTs while many epidermal cells have oblique MTs instead of transverse ones. The slope of MTs becomes steeper in both epidermal and inner tissue cells, as the cells age. It is, therefore, of interest to ascertain whether the orientations of MFs differ between epidermal and inner-tissue cells and also between young and old cells in these organs, in parallel with the orientations of MTs. In this report, we describe an analysis of the arrangement of MFs on the inner surface of the cell walls, using a replica method, and the averaged orientation of MFs, observed under polarized light, in epidermal and inner-tissue cells of

Abbreviations: APM, amiprophosmethyl; MT, microtubule; MF, microfibril.

Avena coleoptiles and mesocotyls and *Pisum* epicotyls.

Materials and Methods

Plants—Avena sativa L. cv. Victory II and *Pisum sativum* L. cv. Alaska plants were grown in the dark at about 27°C, as described previously (Iwata and Hogetsu 1988).

Treatment with APM—Roots and seeds of *Avena* plants which were grown for 2 days in the dark were dipped in water or in solution of 10 $\mu g \cdot ml^{-1}$ APM. The plants were then cultured for 2 days in the dark.

Electron microscopy to examine the arrangement of MFs on the inner surface of the cell wall—Segments excised from various parts of the plants were infiltrated with a solution of 10% glycerol, 0.7 M mannitol, and 0.1% Nonidet P-40, for 10 min. They were frozen, without washing, in a drop of the solution on a freezing stage at about −20°C, and sectioned at an angle to the organ axis at a thickness of 50–60 μm with a sliding microtome, as described previously (Hogetsu and Oshima 1986). The sections were thoroughly washed in distilled water, and placed on a sheet of acetyl cellulose film, which was stuck onto a slide glass, and dried in air. Dried samples were shadowed with platinum-palladium at an angle of 30–45° and coated with carbon. The replicated samples on acetyl cellulose film were cut out with a razor blade and floated on a 70% solution of sulfuric acid for 12 h at 45°C to remove the tissues and the film from the replicas. The replicas were washed three times by transfer onto distilled water with a small piece of coverslip, picked up on copper grids, and examined under an electron microscope (Hitachi 11B; Hitachi Seisakusho Co., Tokyo, Japan).

Polarizing microscopy—Cryosections were mounted in water and the birefringence of cell walls was observed under a polarizing microscope (Olympus IMT-2 equipped with Olympus IMT2-NIC; Olympus Kougaku Co., Tokyo) equipped with a handmade mica compensator (retardation = 1/14 wavelength) for enhancement of contrast. The averaged direction of MFs in the walls was determined by reference to the brightness of end walls in which the preferred direction of MFs is assumed to be transverse to the organ axis. Each specimen was rotated so that the end wall became brightest or darkest. The difference in angles between the two positions represents the angle of the preferred direction of MFs in the cell wall, measured from the direction transverse to the organ axis. To confirm that the contrast observed was not the result of light scattering or absorption, but rather of the birefringence of the material, each section was observed with reciprocal contrast, by rotation of the compensator in opposite directions.

Results

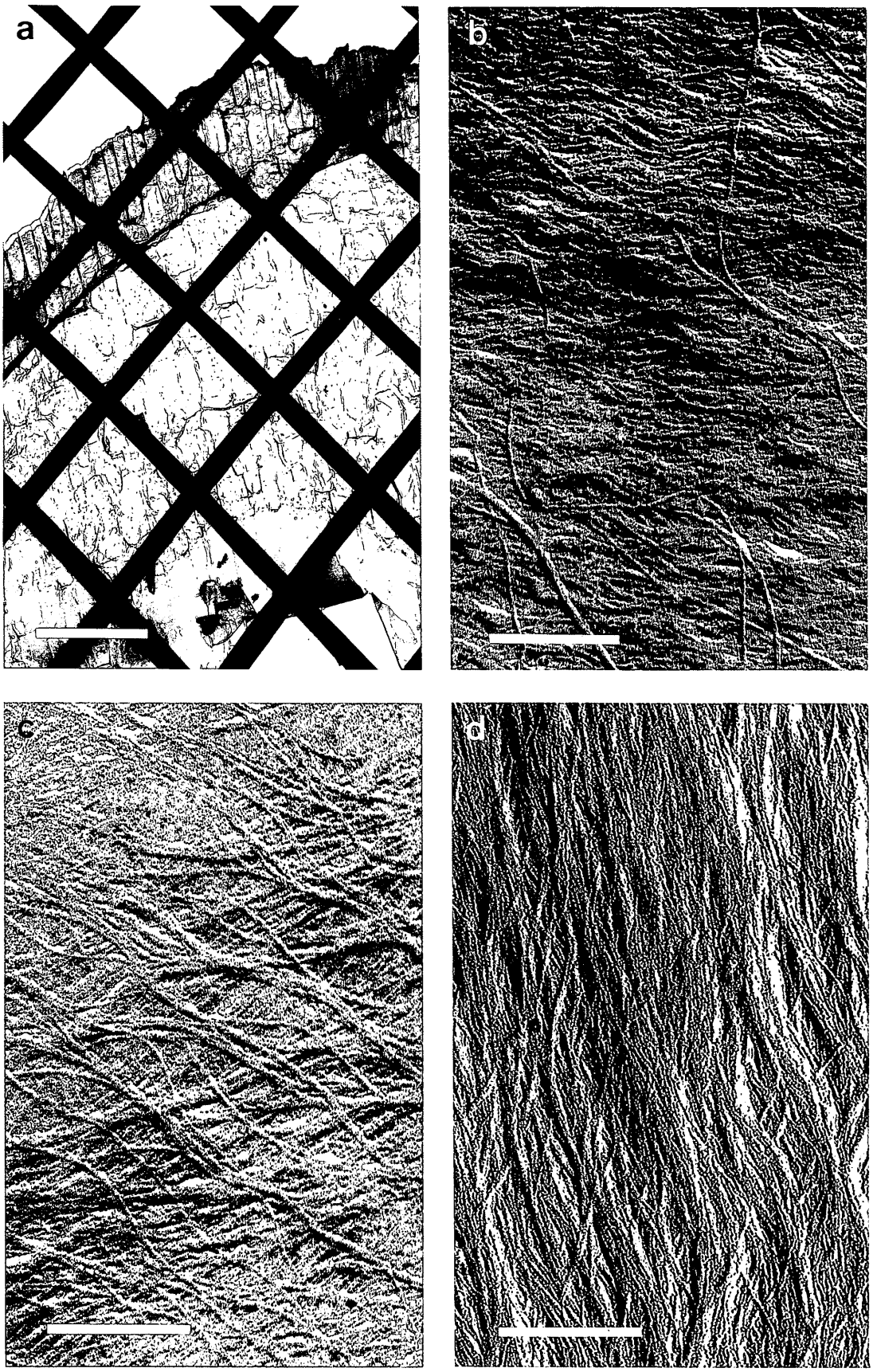
MFs on the inner surface of cell walls—The organ axis was determined from the direction of boundaries between cells which were observed in replicas (Fig. 1). We have here defined the MFs that were predominantly oriented at angles in the range of 70°–90°, 20°–70°, and 0°–20° to the axis as transverse, oblique, and longitudinal MFs, respectively. In coleoptiles (middle part) of 3-day-old *Avena* seedlings, epidermal cells had relatively parallel MFs which were oriented in various directions, while all parenchymal cells had transverse MFs on the inner surface of the cell wall (Fig. 2). We have already reported that cortical MTs are aligned in various directions in epidermal cells, while they are all transverse in parenchymal cells (Iwata and Hogetsu 1988). Thus, the orientations of MFs were similar to those of MTs in both epidermal and parenchymal cells. Both epidermal and parenchymal cells of aged (5-day-old) coleoptiles, in which elongation had already ceased, had longitudinal arrangements of MFs on the inner surface of cell walls (Fig. 2). Such an arrangement of MFs is compatible with an arrangement of MTs in which MTs are oblique in epidermal and parenchymal cells after elongation ceases (Iwata and Hogetsu 1988).

In the elongating region of *Avena* mesocotyls (within 1 mm under the node), MFs were transverse in many epidermal cells, but in a considerable number of epidermal cells, they were oblique (Fig. 3). This pattern is consistent with observations by Takeda and Shibaoka (1981a) that parallel MFs in various orientations are deposited in epidermal cells of Azuki bean epicotyls. MFs in the cortical cells of *Avena* mesocotyls were all transverse. These orientations of MFs are similar to those of MTs (Fig. 3, see also Iwata and Hogetsu 1988).

In the elongating region (within 2 mm under the hook) of *Pisum* epicotyls, MFs were oriented in the same way as those in mesocotyls; in the epidermal cells the MFs were aligned in various directions, while in the cortical cells they were transverse (Fig. 4). MTs in the elongating region of *Pisum* epicotyls are transverse, oblique, or longitudinal in epidermal cells and transverse in cortical cells (Iwata and Hogetsu 1988). Thus, orientations of MFs are also similar to those of MTs in *Pisum* epicotyls.

In aged cells of *Pisum* (49–51 mm under the hook) in the epidermis and cortex, which have stopped elongating, the orientation of MFs on the inner surface of the cell walls

Fig. 1 Electron micrographs of MFs on the inner surface of cell walls. a. Replica of a section of an *Avena* coleoptile at low magnification. b. Transverse MFs on a parenchymal cell wall of an *Avena* coleoptile. c. Oblique MFs on a cortical cell wall of a *Pisum* epicotyl. d. Longitudinal MFs on a cortical cell wall of a *Pisum* epicotyl. Bars = 200 μm (a), 0.5 μm (b, c, d). a, ×90; b, ×43,000; c, d, ×47,500.



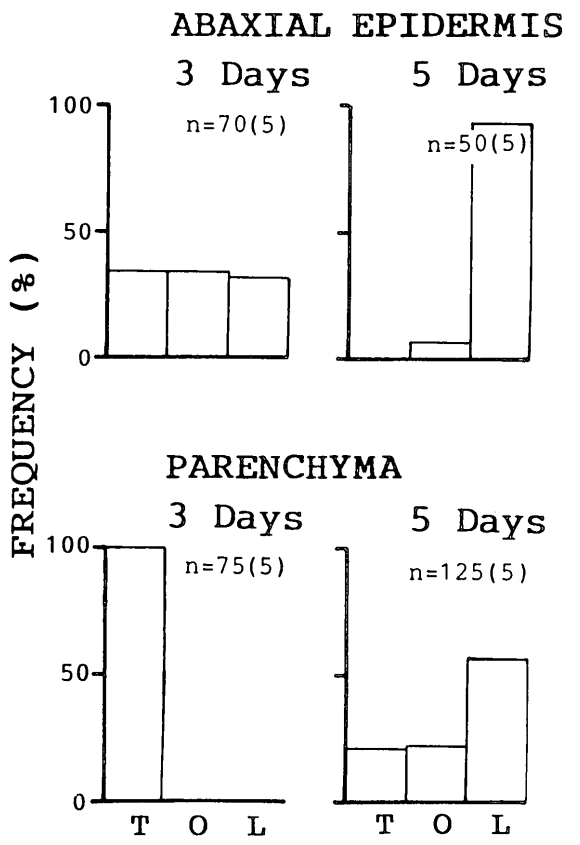


Fig. 2 Obliquity of MFs in *Avena* coleoptiles 3 and 5 days after sowing. T, O, and L indicate cells whose MTs or MFs are transverse, oblique, and longitudinal, respectively. Numbers of examined cells are denoted in the figure as n. Figures in parentheses are numbers of seedlings examined.

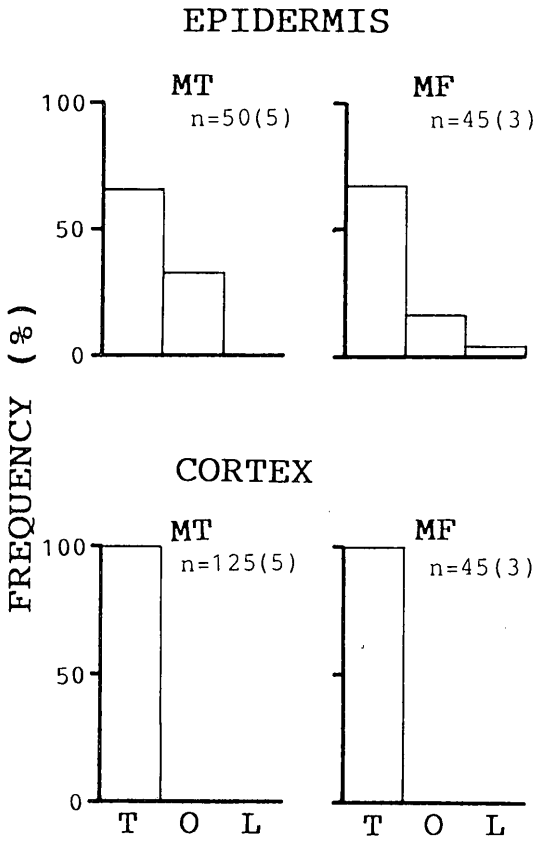


Fig. 3 Obliquity of MFs in *Avena* mesocotyls in elongating portions (0–1 mm under node) 4 days after sowing. See the legend to Figure 2 for details.

was longitudinal (Fig. 4). Such an orientation of MFs is compatible with that of MTs which are oblique or longitudinal in aged, epidermal and aged, cortical cells (Iwata and Hogetsu 1988).

These results are summarized in Fig. 5.

The averaged orientation of MFs—The averaged orientation of microfibrils in cell walls was examined under polarized light. In elongating 3-day-old coleoptiles of *Avena*, MFs in the outer walls of epidermal cells were aligned longitudinally, while those in walls of cortical cells were aligned transversely (Fig. 6, Table 1). Such averaged alignments of MFs in the cell wall were also observed in *Avena* mesocotyls and *Pisum* epicotyls (Fig. 6, Table 1). After elongation ceased, MFs were still longitudinal and

Table 1 Averaged orientation of MFs in the elongating region of each organ

Material		Transverse	Oblique	Longitudinal
Avena coleoptile 3d (2)	Abaxial epidermis	0	0	20
	Cortex	40	0	0
	Adaxial epidermis	20	0	0
Avena mesocotyl 3d (2)	Epidermis	0	0	20
	Cortex	40	0	0
Pisum epicotyl 6d (3)	Epidermis	5	0	31
	Cortex	30	0	0

Figures in parentheses are numbers of seedlings examined.

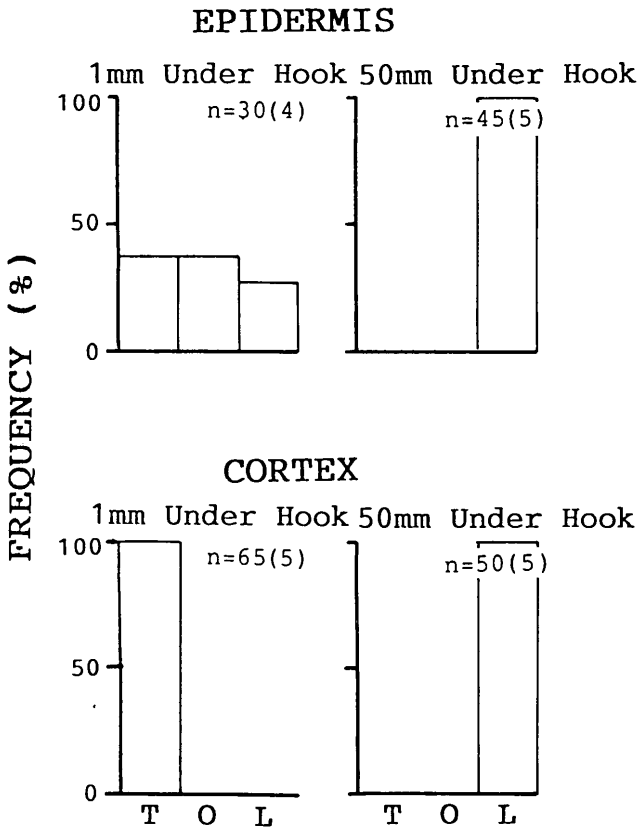


Fig. 4 Obliquity of MFs in *Pisum* epicotyls in elongating (1 mm under hook) and non-elongating portions (50 mm under hook) 6 days after sowing. See the legend to Figure 2 for details.

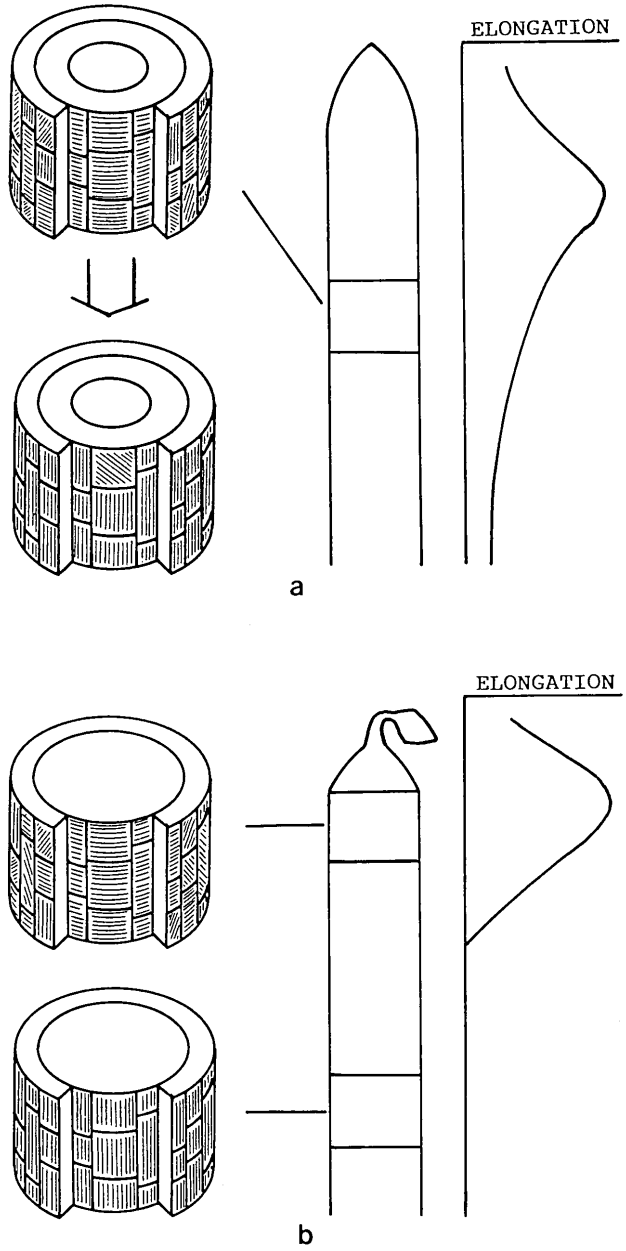


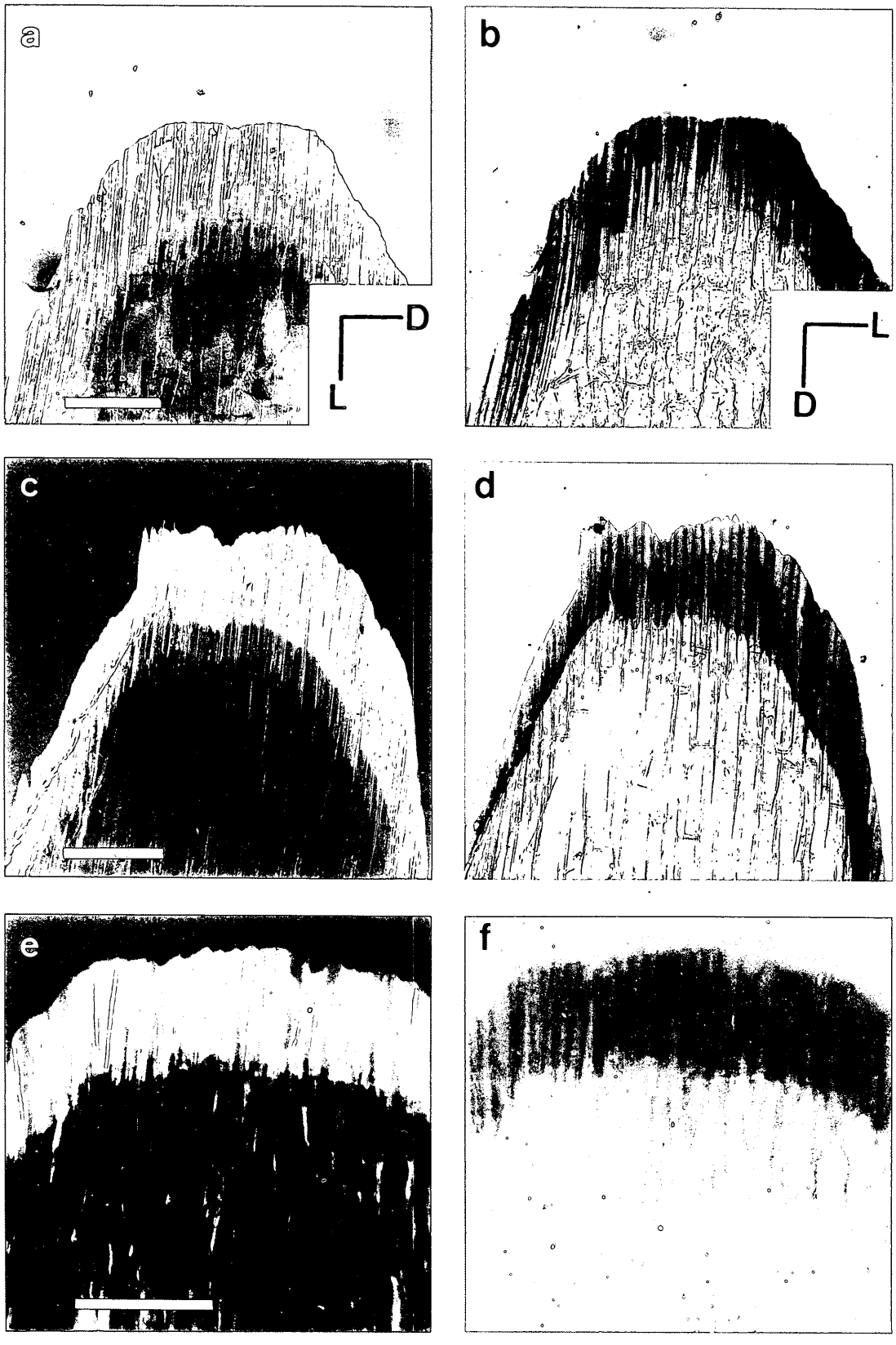
Fig. 5 Diagrams showing the orientation of MFs on the inner surface of walls in cells of (a) *Avena* coleoptile and (b) *Pisum* epicotyl. a. In elongating *Avena* coleoptiles, the orientation of MFs on the inner surface of cell walls of parenchymal and epidermal cells is transverse, and transverse, oblique or longitudinal, respectively. The orientation becomes steeper as elongation ceases. b. In *Pisum* epicotyls, the orientation of MFs on the inner surface of cell walls of cortical and epidermal cells in the elongating region is transverse, and transverse, oblique or longitudinal, respectively. In the non-elongating region, the orientation becomes steeper both in the cortex and epidermis.

transverse, on average, in the outer walls of epidermal cells and in the side walls of cortical cells, respectively, in *Avena* coleoptiles and mesocotyls and in *Pisum* epicotyls (data not shown).

Effects of APM on growth and deposition of MFs in Avena coleoptiles—Disruption of MTs by treatment with $10\,\mu g \cdot ml^{-1}$ APM inhibited the longitudinal growth of *Avena* coleoptiles but promoted lateral expansion (Fig. 7). Areas with different orientations of MFs were found on the inner surface of the cell walls of individual epidermal cells. Areas with different predominant orientations of MFs were also found under polarized light (Fig. 8). In considerable numbers of parenchymal cells in APM-treated coleoptiles, oblique or longitudinal MFs were deposited, while in all parenchymal cells of non-treated coleoptiles deposited transverse MFs were laid down (Fig. 9).

Discussion

We previously reported that cortical MTs in cells of *Avena* coleoptiles and mesocotyls and *Pisum* epicotyls are arranged in a manner specific to the tissues of which the cells are a part and, as elongation ceases, the MTs change their orientation so that they are more closely aligned to the direction parallel to the organ axis (Iwata and Hogetsu



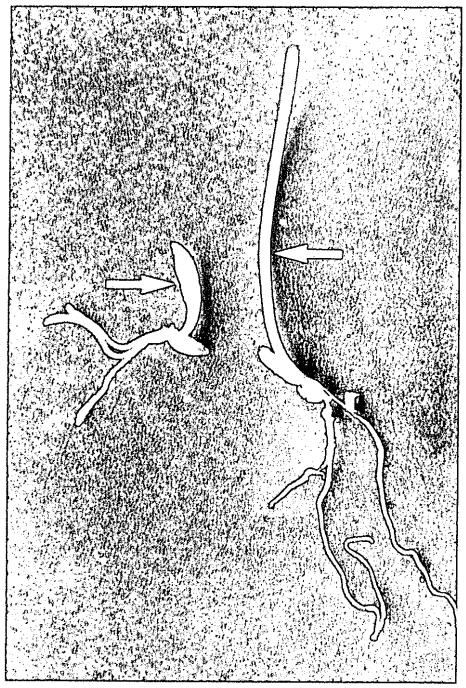


Fig. 7

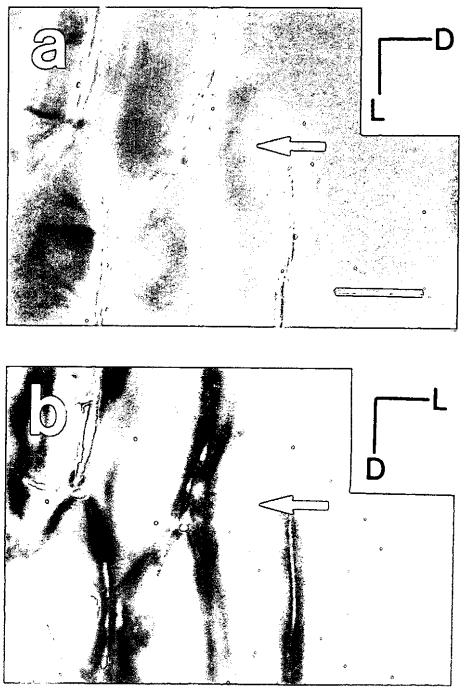


Fig. 8

Fig. 7 Photographs of *Avena* seedlings treated with (left) or without (right) APM at a concentration of $10\,\mu g \cdot ml^{-1}$. Arrows indicate boundaries between the coleoptile and the mesocotyl.

Fig. 8 Micrographs under polarized light of a section from an *Avena* coleoptile treated with APM. A position at which the orientation of MFs is different from that at positions around it, in a single epidermal cell, is indicated by arrows. a, Areas lighter and darker than background have longitudinal and transverse MFs, respectively. b, Areas lighter and darker than background have transverse and longitudinal MFs, respectively. Bar = 20 μm; × 550. See the legend to Figure 6 for the relationship between shades of grey and orientation of MFs.

1988). In the present study, we showed that the arrangement of MFs on the inner surface of the cell walls differs between tissues and changes as elongation ceases, being compatible with the arrangement of MTs. We also showed that the disruption of MTs by the treatment with APM does not influence the parallel alignment of MFs but changes the orientation of MFs. Similar observations have been reported for other plant species (pine cells, Itoh 1976, *Vigna* epicotyls, Takeda and Shibaoka 1981b). These results support the hypothesis that MTs are usually co-aligned with MFs in plant organs and are involved in controlling the orientation of MFs.

Since parallel arrangements of MFs may be much more resistant to mechanical forces along their direction of orientation than to forces perpendicular to it, orientations of MFs in organs may influence the mechanical anisotropy of the walls of constituent cells and, thereby, influence the overall mechanical properties within the organ. In *Pisum* root (Hogetsu and Oshima 1986, Hogetsu 1986) and *Vinca* shoot apex (Sakaguchi et al. 1988), a correlation was observed between the direction of organ growth, which may be determined by the overall mechanical properties of the organ, and the orientations of MFs in the cells of the particular organ.

If MFs facilitate the elongation of cells in the direction perpendicular to them, as is widely believed, the inner tissue and epidermis of *Avena* coleoptiles and mesocotyls and of *Pisum* epicotyls would be reinforced in a transverse and a relatively longitudinal direction, respectively, such that an elongating tension and a contractile force, respectively, would be generated. Such forces have actually been reported in several elongating organs (Kutschera et al.

Fig. 6 Micrographs of sections under polarized light. a, b, A section of an elongating *Avena* coleoptile (3 days old). c, d, A section of an elongating *Avena* mesocotyl (1 mm under the node). e, f, A section of an elongating *Pisum* epicotyl. a, c, e, Cells lighter and darker than background have longitudinal and transverse MFs, respectively. b, d, f, Cells lighter and darker than background have transverse and longitudinal MFs, respectively. The relationship is given in the lower right corner of the Figure a, b. L, lightest; D, darkest. Bars = 200 μm (a, b, c, d, e, f). a, b, c, d, × 80; e, f, × 110.

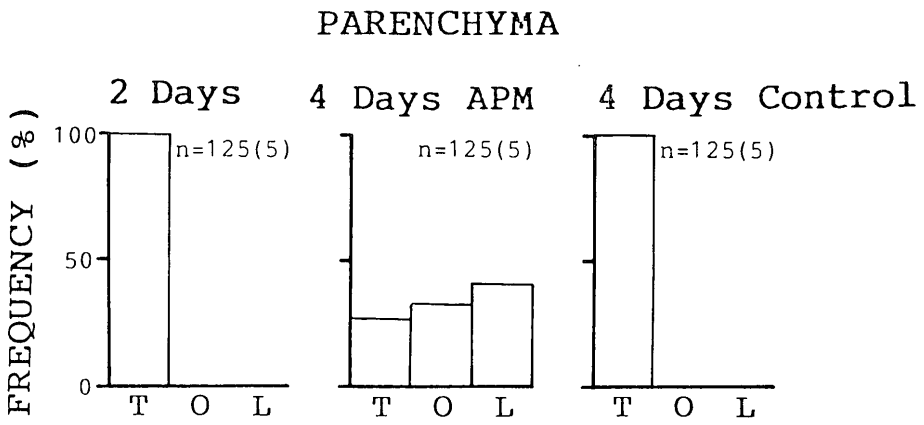


Fig. 9 Effect of APM on the orientation of MFs in parenchymal cells of *Avena* coleoptiles. See the legend to Figure 2 for details.

1987, for review; Kutschera and Briggs 1988). Kutschera et al. (1987) reported that, in *Zea* coleoptiles, an elongating tension in the inner tissues is balanced by the contractile force in the abaxial epidermis. The difference in mechanical properties between epidermis and inner tissues may be derived, at least in part, from the different arrangements of MTs and MFs. The difference in arrangements of the MTs and MFs among tissues was also found in shoot apices (Sakaguchi et al. 1988a). The next and interesting problem to be solved concerns the mechanism responsible for these differences.

The averaged orientations of MFs, as revealed by polarized light, differed between the walls of epidermal and inner-tissue cells, being longitudinal and transverse, respectively. There is a difference between the averaged orientations of MFs, and the orientation of MFs on the inner surface of the cell walls of the epidermis. Almost all epidermal cells have a longitudinal averaged orientation of MFs, although a considerable number of cells have transverse MFs on the inner surface of the cell wall. Possible reasons for this difference may be (1) that MFs in the cell wall are displaced towards the longitudinal direction as the elongation of cells proceeds as suggested by the multi-net theory (Roelofsen 1965), and (2) that the orientation of deposition of MFs on epidermal cell walls changes periodically, and the longitudinal population of accumulated MFs becomes larger than the transverse one. The correct reason remains to be determined.

There is another difference between the arrangement of MFs on the inner surface of cell walls and the averaged orientation of MFs. In non-elongating, inner-tissue cells, the averaged orientation of MFs is transverse, while the arrangement of MFs on the inner surface is oblique or longitudinal. In *Closterium*, the deposition of MFs is known to dwindle to zero after elongation ceases and the secondary wall begins to be deposited (Hogetsu and Takeuchi 1982). A possible explanation for this difference may be that the amount of oblique and longitudinal MFs that must be deposited is so small that the averaged orientation of MFs does not change from transverse to longitudinal.

In conclusion, epidermal and inner tissue cells of *Avena* coleoptiles and mesocotyls and of *Pisum* epicotyls have differently oriented MFs on the inner surface of their cell walls, as is also the case for the respective cortical MTs. The average orientation of MFs also differs between epidermal and inner-tissue cells, being longitudinal in epidermal cells and transverse in inner-tissue cells.

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