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NOTE

Osamu Furusawa · Ryo Funada · Yutaka Murakami
Jun Ohtani

Arrangement of cortical microtubules in compression wood tracheids of *Taxus cuspidata* visualized by confocal laser microscopy*

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Abstract Cortical microtubules (MTs) in differentiating compression wood tracheids of *Taxus cuspidata* stems were visualized by confocal laser microscopy. They were oriented obliquely at an angle of about 45° to the tracheid axis during formation of the secondary wall. Artificial inclination altered the pattern of alignment of MTs. Banding MTs were helically oriented late during the formation of the secondary walls. These results indicate that MTs might control the orientation and localized deposition of cellulose microfibrils in the secondary walls of compression wood tracheids.

Key words Compression wood tracheids · Confocal laser microscopy · Cortical microtubules · Secondary wall · *Taxus cuspidata*

Introduction

Many investigations in a wide variety of plant cells have shown that the orientation of cortical microtubules (MTs) is parallel to that of newly deposited cellulose microfibrils (MFs). Thus, it appears that MTs might control the orientation of newly deposited MFs.[1–4] Various stimuli, such as plant hormones, light, wounding, and gravity, can shift the orientation of MTs in elongating cells.[5,6] Such shifts of MTs should affect the orientation of MFs, with resultant changes in cell morphology. There have been only a few reports about the effects of stimuli on the alignment of MTs in secondary walls, particularly in woody plants.[7–10]

In coniferous trees, compression wood is formed on the lower side of inclined stems or branches, and it functions to maintain the position of the stem or branch or to restore the organ to its original position in space. Compression wood tracheids generally have a specific structure, with a rounded profile, intercellular spaces, a relatively thick middle layer (S_2) in the secondary wall with helical cavities and large microfibrillar angles (microfibrils are oriented at an angle of about 45° to the tracheid axis in a Z-helix), and no innermost (S_3) layer in the secondary wall.[11] The compression wood tracheids of *Taxus* and *Torreya*, both of which have helical thickenings in the innermost surface of the cell walls of their normal tracheids, are characterized by helical thickenings but no helical cavities.[12–16] The orientation of the helical thickenings in the compression wood tracheids of *Taxus* is about 45° in a Z-helix and corresponds to the orientation of the MFs of the innermost surface of the S_2 layer.[13,16–18]

Our goal in the present study was to visualize the arrangement of MTs in compression wood tracheids of artificially inclined stems of *Taxus cuspidata* during formation of the secondary wall. If a direct relation between MTs and MFs exists during formation of the secondary wall, we would expect that MTs would reflect the characteristics of the structure of the cell wall in the compression wood tracheids. We also examined the relation between the formation of helical thickenings and the localization of MTs in an attempt to clarify the role of MTs in the localized deposition of MFs.

Materials and methods

On May 25, 1995 some 10-year-old specimens of *Taxus cuspidata* Sieb. et Zucc., grown in the nursery of Hokkaido University in Sapporo, were artificially inclined and fixed at an angle of 30° to the vertical. Sample trees were cut on July 31; and small blocks, containing mature and differentiating phloem, cambial zone cells, and mature and differentiating

O. Furusawa · R. Funada (✉) · Y. Murakami · J. Ohtani
Department of Forest Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-0809, Japan
Tel. +81-11-706-2516; Fax +81-11-736-1791
e-mail: funada@for.agr.hokudai.ac.jp

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xylem, were taken from the lower side of the inclined stems at breast height.

The procedure used for immunofluorescence staining and confocal laser microscopy has been described elsewhere.[19,20] In brief, small blocks were fixed with a mixture of 3.6% paraformaldehyde plus 0.2% glutaraldehyde that contained 10% dimethylsulfoxide and 0.1% Nonidet P-40 in 50 mmol piperazine-*N*′,*N*′-bis(2-ethanesulfonic acid) buffer (pH 7.0), supplemented with 5 mmol EGTA and 5 mmol $MgSO_4$. After overnight fixation at room temperature, each block was sectioned radially at a thickness of approximately 50 μm with a sliding microtome on a freezing stage (MA-101; Komatsu Electronics, Tokyo, Japan). After washing with phosphate-buffered saline (PBS) pH 7.3, each section was incubated with mouse monoclonal antibody against α-tubulin from chicken brain that had been diluted 1:500 in PBSB (PBS containing 0.1% NaN_3 and 1 mg ml^{-1} bovine serum albumin) for 60 min at 25°C. Sections were then washed with PBS and incubated for 60 min at 25°C with fluorescein isothiocyanate-conjugated antibodies against mouse immunoglobulin G (IgG) that had been diluted 1:10 in PBSB. Antibodies used in the experiments were purchased from Amersham Japan Co. (Tokyo, Japan). The sections were mounted on glass slides and observed with a confocal laser scanning microscope (LSM-310 and LSM-410; Carl Zeiss, Oberkochen, Germany). Images were obtained at 1-μm intervals of the excitation with an argon ion laser (488 nm). Each series of confocal images was processed for construction of a single projection image or a pair of stereoscopic images. Images were printed with a digital color printer (UP-D8000; Sony, Tokyo, Japan).

Small blocks were fixed in 3% glutaraldehyde, dehydrated by passage through a graded ethanol series, and embedded in epoxy resin. Transverse sections 1 μm thick were cut and stained with a solution of safranin. These sections were observed with a light microscope (BH-2; Olympus, Tokyo, Japan).

Results and discussion

As shown in Fig. 1, tracheids with a rounded profile, intercellular spaces, and a relatively thick cell wall were observed in transverse sections. These observations showed that formation of compression wood had been induced on the lower side of stems by artificial inclination.

We examined samples by confocal laser microscopy after immunofluorescence staining of MTs, and we were able to

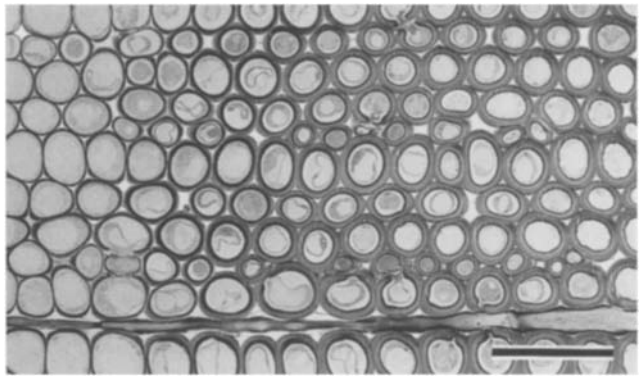

Fig. 1. Light micrograph of a cross section of the differentiating xylem cells in an artificially inclined stem of *Taxus cuspidata*. Tracheids with a rounded profile, intercellular spaces, and relatively thick cell walls can be seen. The left side of this and other micrographs corresponds to the outer side of the tree. Bar = 50 μm

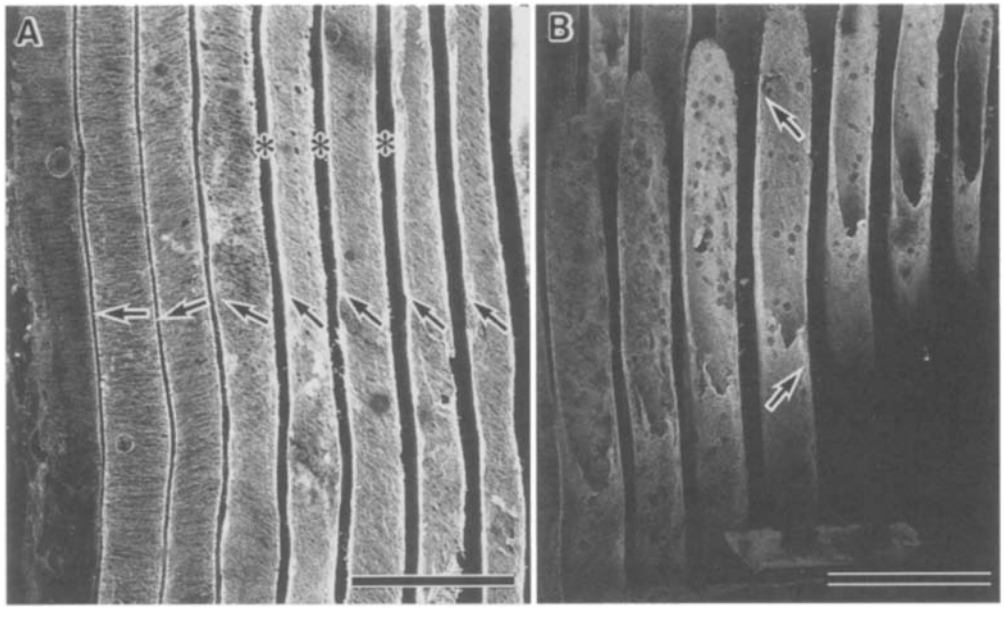

Fig. 2. Images, obtained by confocal laser scanning microscopy, showing the continuous changes in the arrangement of microtubules (MTs) in differentiating tracheids during formation of the secondary wall. **A** Orientation of MTs (*arrows*, viewed from the lumen side) changes from transverse to oblique, with clockwise rotation, at an early stage of formation of the secondary wall. The width of dark regions with minimal fluorescence between the cytoplasm of adjacent tracheids (*asterisks*) increases during differentiation. **B** MTs (*arrows*) are oriented at an angle of about 45° to the tracheid axis in a Z-helix. Bars = 50 μm

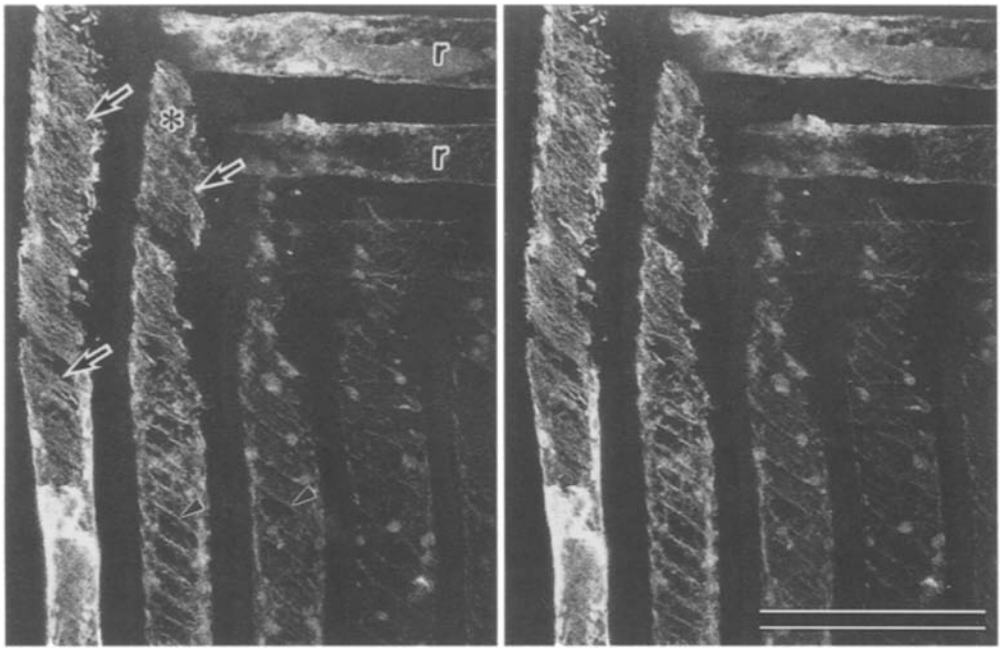

Fig. 3. Pair of stereoscopic images, obtained by confocal laser scanning microscopy, showing the localization of MTs (viewed from the luminal side) in differentiating tracheids at a late stage of formation of the secondary wall. Bands of MTs (*arrows*) are visible, and the helically oriented MTs adopt a rope-like configuration (*arrowheads*). Different arrangements of MTs can be seen within one tracheid (*asterisk*). *r*, ray parenchyma cells. Bar = 50 μm

monitor continuous changes in the arrangement of MTs in differentiating tracheids in a radial file. The MTs in tracheids during formation of the secondary wall after cell expansion were well ordered. During the early stage of formation of the secondary wall, the MTs were oriented transversely with respect to the tracheid axis, and then their orientation changed progressively, with clockwise rotation, as viewed from the luminal side (Fig. 2A). The changes in the orientation of MTs resembled the previously observed changes in tracheids of *Abies sachalinensis* during formation of the secondary wall.[19] Whereas the direction of MTs in normal wood tracheids changed rotatively from transverse to a steep Z-helix, oriented at about 5°–10° to the tracheid axis,[19] the orientation of MTs in compression wood tracheids became oblique until the MTs were oriented at an angle of about 45° in a Z-helix. Approximately 10 tracheids with MTs that were oriented at an angle of about 45° in a Z-helix, with a rounded profile, were observed in a radial file (Fig. 2B). These results showed that the artificial inclination had induced a change in the pattern of alignment of MTs during formation of the secondary wall in tracheids. Obliquely oriented MTs were also found in the developing compression wood tracheids of *Cryptomeria japonica* by electron microscopy.[9] The orientation of MTs in the latter tracheids was consistent with the characteristics of the S_2 layer in compression wood tracheids, with microfibrillar angles of about 45° in a Z-helix.[11] Thus, it is clearly possible that the orientation of MTs is related to that of MFs in compression wood tracheids. The present results support our previous conclusion that a close relation exists between MTs and MFs during formation of the cell wall in the tracheids[19–21] and fibers[10] of woody plants. As the tracheids differentiated, the width of a dark area, with minimal fluorescence, between the cytoplasm of adjacent tracheids (Fig. 2A, asterisks) increased, an indication that the thickness of the cell walls had increased rapidly.

In tracheids that had differentiated still further, the density of the MTs that were oriented at an angle of about 45° in a Z-helix decreased and the arrangement of MTs became slightly disorganized (Fig. 3). At this stage we observed bands of MTs, which were oriented at an angle of about 45° in a Z-helix; they were approximately 3 μm wide (Fig. 3, arrows). In the regions between the bands, we observed slightly disordered MTs that disappeared gradually. The bands of MTs then became narrow and rope-like (Fig. 3, arrowheads). The rope-like bands of MTs were helically oriented underneath the cell wall around the tracheids. When we observed rope-like bands of MTs, helical thickenings were visible under transmitted light with differential interference contrast (data not shown). Uehara and Hogetsu[22] observed similar bands of MTs, superimposed on helical thickenings, in the differentiating normal wood tracheids of *Taxus cuspidata*. These rope-like bands of MTs might be involved in the formation of helical thickenings, and so they might control the localized deposition of MFs. In addition, the angle of the bands of MTs was about 45° in a Z-helix, being the same as the angle of MTs at the earlier stage of differentiation, as shown in Fig. 2B. These results might be related to the fact that the helical thickenings have the same orientation as the innermost layer of the secondary wall in compression wood tracheids.[13,16–18] Different arrangements of MTs were observed within some tracheids (Fig. 3, asterisk). In one part of such a tracheid, MTs were localized in a band-like pattern, and disordered MTs were observed between the bands. By contrast, in an other part of the tracheid, rope-like bands of MTs were observed with

few MTs between the bands. Such a tracheid might be at a transitional stage during the dynamic relocalization of MTs. These results indicate that changes in the arrangement of MTs could occur nonuniformly within a single tracheid, suggesting that the stage of differentiation might differ and might depend on the position within the tracheid. As the tracheids differentiated, the fluorescence of MTs became weaker and finally disappeared altogether. During formation of the secondary wall, the orientation of MTs changed from transverse to an angle of about 45° in a Z-helix, and the same orientation was retained until differentiation was complete. These results are consistent with the absence of the S_3 layer, whose MFs are normally oriented in a flat S-helix, in compression wood tracheids.[11]

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