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EARLY CHANGES IN THE RADIAL WALLS OF STORIED FUSIFORM CAMBIAL CELLS DURING FIBER DIFFERENTIATION

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SUMMARY

There is little information about the ultrastructural changes taking place in the radial walls of fusiform cambial cells during differentiation into xylem derivatives. The present study reports the early events occurring in the radial walls of fusiform cambial cells (FCCs) during fiber elongation in *Holoptelea integrifolia*, a deciduous tropical tree with storied cambium. Serial tangential sections of active cambial zone cells demonstrate the initiation of intrusive cell wall elongation from gabled ends of FCCs during fiber development. The elongation at the tip is followed by the axial extension of the entire cell. It was evident from ultrastructural observations made on the tangential sections that the thick beaded pattern on FCC radial walls disappear following cell elongation. PATAg staining, specific for wall polysaccharides showed that, initially, the beaded structures undergo wall loosening following hydrolysis of pectic polysaccharides in the middle lamella. Then the loosened primary walls come together with the axial extension of cells. Thus the beaded nature disappears in the differentiating cambial cells. This study highlights the cell wall changes associated with the differentiation of FCCs into fibers.

Key words: Cell wall changes, storied cambium, fusiform cells, fiber differentiation, beaded cell walls, *Holoptelea integrifolia*.

INTRODUCTION

Wood is produced as a result of successive centripetal addition of cells from the vascular cambium. The cambial derivatives undergo an ordered developmental process before developing into wood elements (Mellerowicz *et al*. 2001). Hardwoods are complex with differential arrangement and frequency of vessels, fibers and parenchyma cells. Among the wood elements fibers are the longest cells with narrow pointed tips. Of particular importance are the primary walls of cambial cells which undergo structural, physical and chemical changes before developing into a particular element with a definite structure and function in the wood. The cambial derivatives developing into fibers undergo intrusive growth followed by secondary wall deposition and lignification. There is little information in hardwoods to help us to understand how the cell wall develops during fibre elongation.

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The tangential and radial walls of cambial zone cells have different structural organization. Compared to the tangential walls the radial walls of a fusiform cambial cell (FCC) are relatively thick with several primary pit fields. The radial walls are especially thick between primary pit fields thus giving them a beaded appearance in tangential section (Kerr & Bailey 1934; Catesson 1994). The beaded pattern is more prominent in storied cambium as FCCs are relatively short with no appreciable elongation. However, the beaded cell wall pattern is lost in the wood elements derived from FCCs. The thick radial walls of dormant cambial zone cells become thin with the onset of cambial activity. The thinning of radial walls is known from ultrastructural studies of transverse sections of cambium (Catesson *et al*. 1994). However, there is little information on how the beaded structures observed in tangential view of radial walls disappear following cambial cell differentiation.

Moreover, most structural and biochemical studies on the cambium have been carried out in species from the temperate zone (Catesson 1994). This study instead made use of a tropical species, *Holoptelea integrifolia,* with storied cambium. The storied cambium is useful because the storied arrangement of cells helps to identify the fiber initials and to study the cell elongation during its differentiation. In *Holoptelea*, the mean length of fibers is 4–4.5 times greater than the mean length of fusiform initials (Rao *et al*. 1989). The goal of this study is to characterize the early events leading to the elongation of FCC and the disappearance of beads in the cambial zone during fiber differentiation in the tropical hardwood using both light microscopy and transmission electron microscopy (TEM).

MATERIALS AND METHODS

Plant material

Differentiating cambial cells were collected in September 2004 along with the outermost xylem from the main trunk at chest height of *Holoptelea integrifolia* Planch. trees growing in the dry deciduous forest (Jambughoda) of Gujarat state, India. The information available on the seasonal cambial growth pattern in *Holoptelea* (Rao & Dave 1984) helped us to know the appropriate month for collection of active cambium samples with 5–8 layers of differentiating xylem which is suitable for the present study.

Light microscopy

Light microscopy was used to follow the morphological changes in the cambial derivatives. For light microscopy, the tissues were fixed in formalin-acetic acid-ethyl alcohol (FAA) (Berlyn & Miksche 1976) and embedded in paraffin. Serial tangential sections from the cambial zone towards xylem were made with these paraffin embedded samples. They were then stained with tannic acid-ferric chloride-lacmoid (Cheadle *et al*. 1953), which stains cambial cell walls and was useful for this study because it precisely contrasts cell walls rather than cytoplasm. Samples were then observed at ×40 magnification, paying particular attention to growing tips of fiber initials. The study was not quantitative; the goal was to make observations of general temporal patterns

that we could deduce from the position of the sample relative to the cambium. Photographs were taken with Image pro-plus (Media Cybernetics). A second set of samples was used for ultrastructural studies. The samples were immediately fixed in 2.5% glutaraldehyde in 2% paraformaldehyde and then trimmed into 1–2 mm size pieces after coming to the laboratory and left in fresh fixative for 3 hours followed by 2% osmium tetroxide overnight. After the routine dehydration and infiltration the samples were embedded in Epon. Semithin sections on glass slides were stained in 1% toluidine blue to locate and reach the cambial zone by the removal of xylem and phloem tissue surrounding it.

Transmission electron microscopy

For transmission electron microscopy (TEM), ultrathin sections of cambial zone cells were taken from isolated tissue. Sections were made using a diamond knife and placed on nickel and gold grids and then subjected to periodic acid-thiocarbohydrazide-silver proteinate (PATAg) (Thiery 1967). This staining visualizes cell wall polysaccharides. Samples were observed with a Jeol 1212 TEM. The cell wall features such as presence/absence of beads, their opacity vs. transparency, the apparent shape of the layers in the cell wall, and loosening of the cell wall materials and elongation were observed.

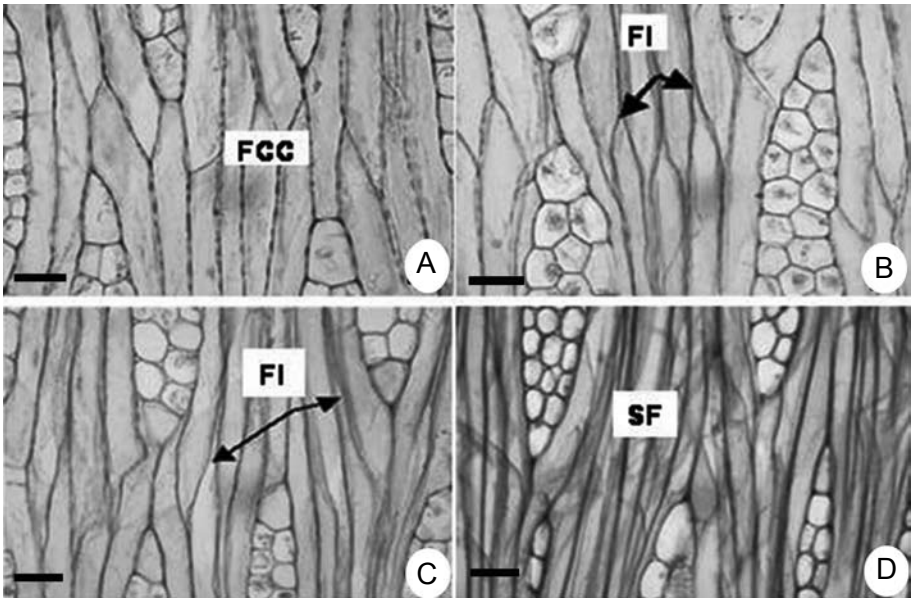

Figure 1. Tangential sections obtained from cambial zone inward towards xylem from trunk wood of *Holoptelea integrifolia* observed with light microscopy (tannic acid-ferric chloride-lacmoid staining). – A: Storied arrangement of fusiform cambial cells (FCC). Note the gabled cell ends at the same level. – B: Fiber initials (FI) showing elongation of gabled cell tips (arrows). Compare the cell tips of fiber initials with those of FCC shown in 'A'. – C: Fiber initials (FI) showing further elongation at the intrusively growing tips (arrows). – D: Fibers with secondary walls (SF) after complete elongation. — Scale bar = 10 μm.

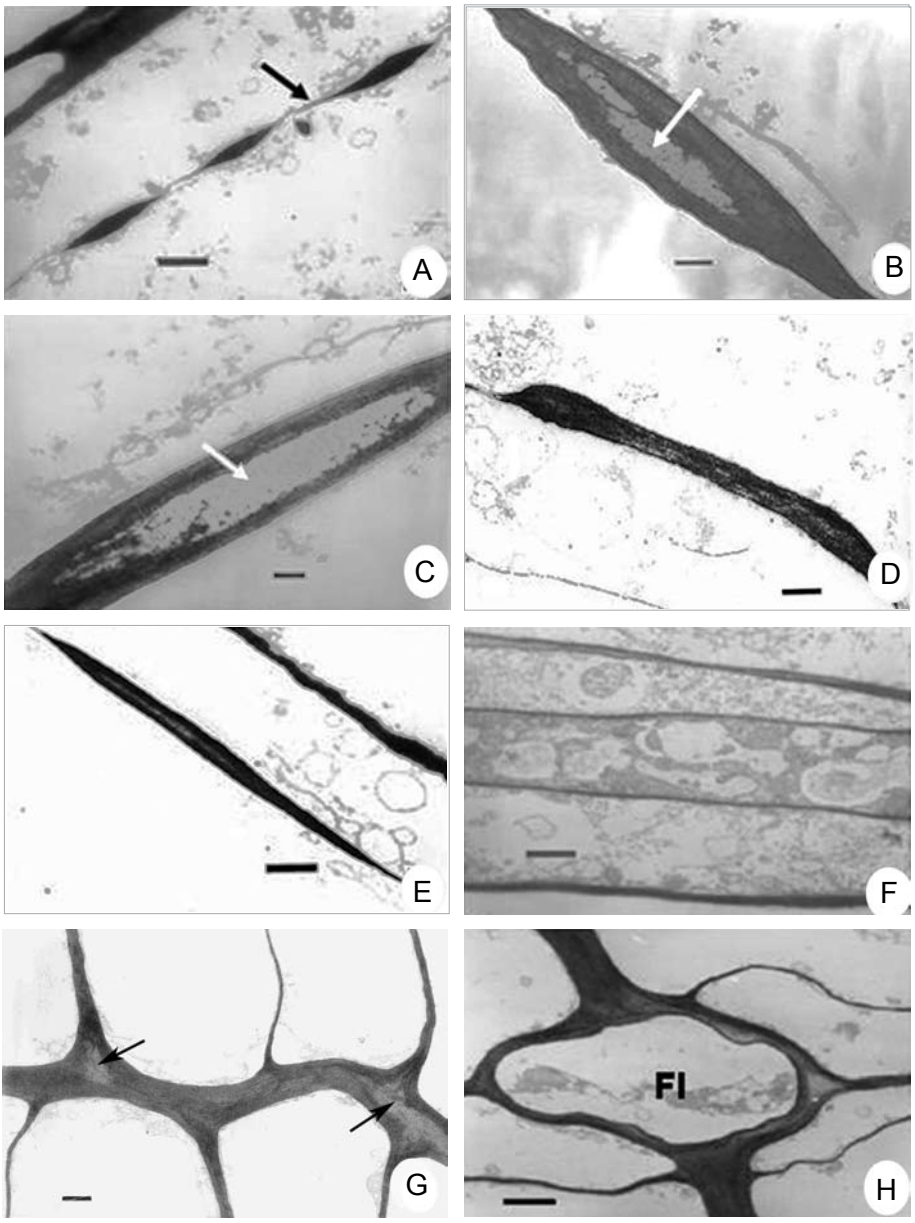

Figure 2. Tangential (A–F) and transverse (G & H) sections of fusiform cambial cells observed under TEM following PATAg staining. – A: Darkly contrasted radial cell wall showing beaded regions separated by thin primary pit-fields (arrow). – B: A bead with central less electron dense region (arrow) indicating the loosening of middle lamella between the adjacent primary walls. – C: Further loosening of middle lamella extending axially along the cell wall. – D: The separated primary walls remain together following the stretching of the walls during intrusive growth. – E: Thinning of the beaded wall portion following cell elongation. Note the remains of the

RESULTS

In *Holoptelea* the cambial zone is composed of axially elongated storied fusiform cells (FCCs) and short isodiametric ray cells. The FCCs, arranged in definite horizontal rows are uniform in size with abrupt ends occurring nearly at the same level (Fig. 1A). The centripetal derivatives of FCCs give rise to vessel elements, fibers and axial parenchyma. Both the vessel elements and axial parenchyma retain their storied arrangement in the mature xylem, as seen in tangential longitudinal sections.

Fiber initials can be identified very early among the cambial derivatives (Fig. 1B). The serial tangential sections obtained in the centripetal direction from the cambial zone revealed stages in the differentiation of fibers from FCCs. The fibers, however, soon become nonstoried with their ultimate length several times longer than that of FCCs. In transverse sections we have encountered fiber cell differentiation closer to the cambial zone as shown in Figure 2H. It appears from the sections that the elongation of cell ends starts simultaneously at the overlapping region between the vertically adjacent rows of FCCs. In the initial stages of fiber development the narrow cell tip derived from the gabled end of FCCs intrude between the middle lamella of adjacent cells (Fig. 1C). However, closer to the cambial zone only one or two cells from the FCC tier reveal the tip elongation which is also identified from transverse sections (Fig. 2H). The initial tip elongation is followed by elongation of the entire cell and secondary wall deposition (Fig. 1D).

The early radial wall changes occurring in the FCC derivatives developing into fibers were followed under TEM. In tangential sections the radial walls of FCCs appear beaded, each bead representing thickened primary walls of adjacent cells enclosing the middle lamella. The adjacent beads are separated by thin primary wall areas representing primary pit-fields traversed by several plasmodesmatal connections. However, this beaded pattern is not found on the tangential walls which, unlike radial walls, are laid down following each periclinal cell division. The beaded portions of the cell wall within the cambial zone appear darkly contrasted when stained with PATAg, a specific stain for cell wall polysaccharides under TEM (Fig. 2A).

During cambial cell differentiation the walls are stretched in radial, tangential and axial direction depending on the kind of developing xylem element. This results in the disappearance of beaded structures and many of the primary pits from the radial walls. Subsequently the radial walls appear more or less uniformly thick. In *Holoptelea*, the axially elongating FCC derivatives on the xylem side develop into fibers. In the differentiating cells beaded wall material becomes less dense following the dissolution

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beaded wall closer to the primary pit-fields. – F: Elongating fiber initials showing uniformly thick radial walls following the disappearance of beaded structures. – G: Cambial zone radial walls showing loosening of the middle lamellae (at arrows). – H: A fiber initial (FI) cell tip intruding through the radial walls of adjacent radial files of cambial zone. Note the recently laid down thin tangential walls closer to the fiber initial. — Scale bar for A & F = 2 μm; for B & C = 500 nm; for D, E, G, H = 1 μm.

of the middle lamella portion of each bead and allows visualization of the typical three-layered cell wall structure: primary wall, middle lamella and primary wall (Fig. 2B). The matrix of the middle lamella is known to be composed of acidic pectins. Subsequently the primary wall of each side containing densely packed microfibrils could easily be discerned as distinct separate layers in each bead (Fig. 2C). Following intrusive growth of fiber initials the beaded area becomes stretched resulting in their disappearance on the radial walls (Fig. 2D & E). New wall material may then be deposited thus permitting the increase in the cell wall area in axially growing fiber initials (Fig. 2F). Transverse sections of the cambial zone also revealed radial walls with loosely organized wall materials (Fig. 2G), probably corresponding to the loosening of beaded wall as observed in tangential sections (Fig. 2C). Transverse portions of intrusively elongating fiber initials are often noticed between the adjacent radial files of FCCs (Fig. 2H).

DISCUSSION

Holoptelea integrifolia is a deciduous tropical species with storied cambium showing distinct seasonal changes (Rao & Dave 1984). The present study mainly highlights the initial cell wall changes associated with the elongation of FCC derivatives into fibers closer to the cambial zone. In tangential sections FCCs are identified with certainty from the differentiating cells due to their beaded radial walls. As the cambial zone is narrow between the massive xylem and phloem tissues in a tree trunk, it is obviously difficult to observe true cambial cells from the tangential sections. The serial tangential sections taken in centripetal direction of the cambial zone reveal the gradual changes occurring in the cell walls of FCCs during differentiation into fibers.

The light microscopic observations reveal the elongation of the wall at the abrupt cell ends which penetrate the middle lamella of the neighboring cells. The initiation of cell elongation commences at the gabled ends of the FCCs. Cells with such elongating tips can decisively be identified as fiber initials in tangential sections. On the other hand, the cells with narrow transverse diameter between the radial files of FCCs at the centripetal margin of the cambial zone are identified as elongating fiber initials in transverse sections. Thus the radial seriation in the cambial zone cells is disturbed by the elongating fibers. There is a good reason why the intrusively growing cells close to the cambial zone will develop into fibers rather than other cell types in trees with storied cambia: unlike developing parenchyma strands and vessel elements they loose their storied arrangement during elongation. Fibers are the longest cells among all cell types of secondary xylem and they will probably take more time for their elongation and maturation than vessel elements and parenchyma strands. Earlier studies indicate that vessels differentiate very close to the cambial zone and it is considered that vessel elements are the first cells to differentiate from cambium (Larson 1994). The present study convincingly demonstrates that fibers begin to differentiate at least as close to the cambial zone as vessels. Ultrastructural information on cambial cell walls mainly comes from investigations focused on transverse sections covering both the tangential and radial walls. Changes associated with radial walls of FCCs during the onset of

activity have been described in temperate tree species (*e.g.* by Funada & Catesson 1991). It is obvious from the cambial studies that the characteristic beaded pattern of radial walls known from tangential sections cannot be discerned from the transverse sections of cambium.

The loosening of radial walls is an obvious and well established phenomenon reported from transverse sections of differentiating cambial cells (Funada & Catesson 1991; Catesson 1994; Catesson *et al*. 1994). However, the initial radial cell wall changes occurring in FCCs using tangential sections are not reported. When growth is active with cell hydration and swelling, the walls become thinner and the primary pit fields are drawn further apart (Fahn & Werker 1990). The variation in the wall topochemistry and structure between tangential and radial walls was demonstrated through cytochemical investigation coupling mild extractions with specific staining (Roland 1978; Catesson & Roland 1981). On the other hand cambial cell wall loosening requires the cleavage of cross linked polymers through wall bound esterases, glycans or proteases (Catesson 1990) and breakdown of calcium-linked pectin molecules (Funada & Catesson 1991). Cell expansion in the cambial zone is thought to be driven by turgor pressure and facilitated by auxin promotion of cell wall loosening (Savidge 1996). The present study clearly demonstrates the loosening of beaded walls of FCCs at the time of cell elongation in the cambial zone. The cell tip elongation of storied FCCs of *Holoptelea* is considered to arise in response to axial polar flow through the cambial zone. Two possible mechanisms of intrusive growth are cell elongation by growth of the very cell tip: cell tip growth; and cell elongation by growth along the surface of the entire cell: diffuse growth (Ageeva *et al*. 2005). In *Holoptelea*, fiber initials elongate at the tips facilitating the cell tips to intrude into the middle lamella of neighboring cells followed by elongation of the entire cell. This intrusive growth pattern during the early growth stage has been evident from the greater mean length of fibers than that of fusiform intials in *Holoptelea* (Rao *et al*. 1989). The cell wall changes observed in the present study may be similar to those made on the transverse sections of cambium (Catesson 1994; Funada & Catesson 1991). On the other hand, a comparison between fibers and vessel elements at early stages of differentiation could be interesting as the former undergoes marked elongation with limited enlargement and the latter shows a spectacular increase in diameter with little or no elongation.

In conclusion, our ultrastructural findings for the first time conclusively demonstrate how the classical beaded nature of FCC radial walls disappears during cambial cell differentiation. It is evident from light microscopic data that intrusive growth is initiated at the gabled ends of storied FCCs. It is often difficult to identify the early stages of fiber differentiation in species with nonstoried cambium, as all the elongating cambial derivatives already possess gradually tapered overlapping cell ends. While those with storied cambium fiber initials can be easily recognized at a very early stage through elongation of their cell tips. Therefore, storied cambium can be used as a model for understanding the fiber differentiation process in woody plants. On the other hand, a comparison of cell wall changes occurring between different cell types at their early stages of differentiation from cambium could also be interesting.

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