CELLULAR CHANGES OF TRACHEIDS AND RAY PARENCHYMA CELLS FROM CAMBIUM TO HEARTWOOD IN *CUNNINGHAMIA LANCEOLATA*

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SONG K, LIU B, JIANG X & YIN Y. 2011. Cellular changes of tracheids and ray parenchyma cells from cambium to heartwood in *Cunninghamia lanceolata*. Cellular changes of the cell wall and protoplasm in tracheids and ray parenchyma cells during heartwood formation were investigated in a 26-year-old *Cunninghamia lanceolata* at micro- and ultrastructure levels. Cell morphological and ultrastructural features changed significantly from the cambium to inner heartwood. In the cambial zone, the thickness of fusiform and ray cell walls were thin and their radial walls were much thicker than tangential walls. Fusiform cells were highly vacuolated with the protoplasm confined to the periphery of cell lumen. At the time of wood cell differentiation, differentiating xylem mother cells began to lose the protoplasm. Concomitantly, their walls thickened and showed characteristically distinct wall layers. In comparison, ray cell walls appeared as typical polylamellate structure and had thinner walls than the tracheids. Tracheids completed their differentiation and left hollow dead tracheary elements to decline into sapwood, while the xylem ray cells remained alive. The ray cells contained cell protoplasm, although the amount, shape and size altered when shifting towards intermediate wood. Subsequently, the ray cells disintegrated their protoplasm, including the nuclei, organelles and reserve materials, which marked the formation of heartwood.

Keywords: Ultrastructural features, cell wall, protoplasm, heartwood formation, xylem ray cells

SONG K, LIU B, JIANG X & YIN Y. 2011. Perubahan sel trakeid dan sel parenkima ruji dari kambium ke kayu teras dalam *Cunninghamia lanceolata*. Perubahan peringkat sel pada dinding sel dan protoplasma dalam trakeid serta sel parenkima ruji ketika pembentukan kayu teras diselidiki pada *Cunninghamia lanceolata* yang berusia 26 tahun secara mikrostruktur dan ultrastruktur. Ciri-ciri morfologi serta ultrastruktur sel berubah secara signifikan dari kambium ke bahagian dalam kayu teras. Dalam zon kambium, dinding sel fusiform dan sel ruji adalah nipis dan dinding jejari jauh lebih tebal daripada dinding tangen. Sel fusiform dipenuhi vakuol dan protoplasma terhad kepada pinggir lumen sel. Ketika pembezaan sel kayu, sel induk xilem yang sedang mengalami pembezaan mula kehilangan protoplasma. Seiring dengannya, dinding sel juga bertambah tebal dan menunjukkan lapisan dinding yang jelas. Sebaliknya, dinding sel ruji nampak seperti struktur berlamela yang banyak dengan dinding yang lebih nipis berbanding trakeid. Apabila pembezaan trakeid selesai unsur trakeid yang mati dan lompang kekal dalam kayu gubal lalu menyusut. Sebaliknya sel ruji xilem terus hidup. Sel ruji xilem mengandungi protoplasma sel namun jumlah, bentuk serta saiznya berubah apabila menjadi kayu perantaraan. Akhirnya, sel ruji menguraikan protoplasmanya termasuk nukleus, organel dan bahan simpanan. Ini menandakan bermulanya pembentukan kayu teras.

INTRODUCTION

The secondary vascular system in trees is a dynamic developmental process and consists of secondary phloem, vascular cambium and secondary xylem. Secondary xylem is the product that differentiate from the cambium and contains various types of xylem cells with different functions. Transformation of wood from sapwood to heartwood is a complex developmental biological process, which is an essential part of wood secondary growth. It is the most important physiological phenomenon after cambial initials are differentiated to xylem cells (Savidge et al. 2000). Moreover, heartwood can help tree trunks increase their durability, resistance from microbial invasion and structural support (Bamber & Fukazawa 1985, Hillis 1987).

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From the cambium to xylem, fusiform cells lose their nuclei and cell contents immediately, leaving hollow dead cells that form tracheary elements. By contrast, xylem ray parenchyma cells which play an important role in the storage and radial transport of materials remain alive for several years even after the completion of secondary wall formation and lignification (Funada 2000, Nakaba et al. 2006, Spicer & Holbrook 2007). This indicates that the differentiating process of ray parenchyma cells is separated from that of tracheary elements. The vitality of ray parenchyma cell, whose death is proposed to mark the transformation of sapwood into heartwood, has been recorded by the cytological changes of cytoskeleton, vacuoles, organelles, storage materials (Nakaba et al. 2008) and cell wall (Pandalai et al. 1985).

Heartwood formation is a unique biological process in trees. To date, numerous features during the transformation from sapwood to heartwood have been widely studied, including seasonal features, genetic expression, composition of gases, water distribution, chemical composition, enzyme activity, structural alterations in shape, size, colour and natural durability. However, the mechanisms of transformation of xylem cells during heartwood formation have not yet been completely clarified. Particularly, fewer details of cellular changes concerning cell wall and protoplasm of ray parenchyma cell during the death of xylem have been characterised than that of tracheid elements in the tree system. Further understanding of this unique feature, i.e. heartwood formation in trees may enable duplicating them in bio-inspired polymer composites.

Therefore, the main objective of this study was to investigate the characteristics of cellular changes among cambium cells, the immature xylem cells adjacent to cambium and mature xylem cells within sapwood and heartwood, in regard to tracheids and ray parenchyma cells in *Cunninghamia lanceolata* (Taxodiaceae) by direct observations. This species is known for its high ratio of heartwood and very good durability. It is easy to determine the boundary between sapwood and heartwood because of its distinct and wide growth rings. Moreover, it is one of the most important commercial softwood plantation species in southern China. Since the rays in *C. lanceolata* are composed of parenchyma cells without any ray tracheids, it is easy to distinguish cellular changes in the ray parenchyma cells during their transformation into heartwood. In this study, the thickness and structure of the cell wall in tracheids and ray parenchyma cells, shape and size of nuclei, cell organelles and storage materials in ray parenchyma cells were determined to better understand the process of heartwood formation which plays a considerable role in tree breeding and wood utilisation.

MATERIALS AND METHODS

Plant materials

A vigorous *C. lanceolata* (clone Long-15) tree of approximately 26 years of age grown in the Longshan Forest Center of Zhejiang Province in China was felled for this study. Immediately, small blocks from five different regions, i.e. wood adjacent to cambium, middle sapwood, intermediate wood, middle heartwood and inner heartwood were obtained along the radial direction from both halves of the main stem (Figure 1). It was easy to identify the intermediate wood (the boundary between sapwood and heartwood) based on colour variations. The middle sapwood was located in the middle between cambial zone and intermediate wood. The middle heartwood was located in the middle between the intermediate wood and the pith. The inner heartwood was near to the pith. After sampling positions were determined, the number of growth rings of each sampling position was counted from the pith to cambium (Table 1). These samples were all excised at breast height (1.3 m).

Light microscopy

For the preparation of 2 µm thick transverse sections, wood samples were immersed immediately after collection in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After returning to the laboratory, these blocks were cut into small slivers (1 × 1 × 5 mm) containing earlywood, and then placed in fresh fixative under slight vacuum for 30 min. Following vacuum treatment, these pieces were fixed in fresh fixative for four hours at room temperature. Thereafter, they were washed four times with the same buffer and post-fixed in 1% osmium tetraoxide in 0.2 mol l^{-1} buffer overnight at 4 °C in a refrigerator.

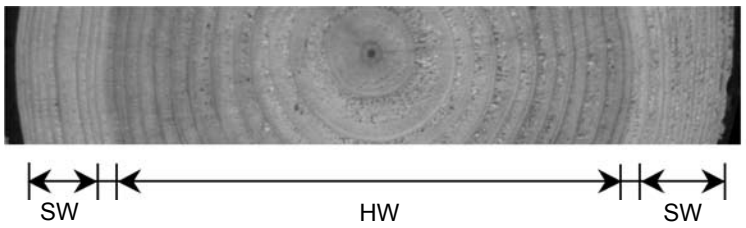

Figure 1 A sample wood disc of *Cunninghamia lanceolata* showing the distribution of sapwood (SW) and heartwood (HW). Intermediate wood is shown as paler-coloured xylem between the sapwood and heartwood.

Table 1 Radial positions of wood sample collection

Sampling position	Cambium zone	Middle sapwood	Intermediate wood	Middle heartwood	Inner heartwood
Growth ring	26	18–21	13–14	10–11	6–7

Subsequently, the pieces were dehydrated through a graded ethanol series and embedded in Spurr resin. Transverse sections were cut with a glass knife fixed on an ultramicrotome and stained with 1% aqueous solution of toluidine blue for observation under light microscope.

Transmission electron microscopy

The same embedded specimens, which were used for the preparation of 2 µm thick sections, were cut with diamond knife mounted on the ultramicrotome. Sequential ultrathin transverse sections of approximate 60 nm thickness were picked up on formvar coated grids and stained with uranyl acetate and lead citrate (Côté 1965). Features of protoplasm and cell wall were characterised at 80 kV by a transmission electron microscopy. A total of 40 earlywood tracheids of each sample were randomly selected to measure the thickness of radial and tangential walls. Additionally, five ray parenchyma cells in earlywood from each sample were randomly selected for thickness measurement. For each parenchyma cell, the thickness at three positions was randomly measured on both the radial and tangential walls. The parameter of nuclear slenderness ratio was calculated as the average length divided by the average width of nucleus in transverse section.

Statistical analysis

Statistical analysis was carried out using SAS program, version 9.0. The effects of radial position from cambium zone to the inner heartwood on the thickness of cell walls, including radial and tangential walls, were analysed by one-way analysis of variance (ANOVA). Multiple comparison method was applied using Fisher's least significant difference test.

RESULTS

Cellular changes of cambial fusiform cells and ray cells

Cell walls of the fusiform cambium cells were very thin and their shapes were irregular (Figures 2a and 3a). Cell wall thickness of the fusiform cambial cells was about 0.76 µm for radial walls and 0.19 µm for tangential walls (Table 2). These values were significantly less than those of xylem tracheids ($p < 0.05$). From the cambium to immature sapwood, differentiating xylem mother cells enlarged radially followed by deposition of secondary wall material; thus the cell walls were becoming thicker (Figure 2a).

Cambial fusiform cells were highly vacuolated, as illustrated by the large central vacuole in most of the cell lumens; so cytoplasm was squeezed with a strip distribution around the periphery (Figures 3a and b). The nuclei were usually oval or elliptical with a lot of organelles surrounding it. Organelles included mitochondria, endoplasmic reticulum (Figure 3b and c) and Golgi apparatus.

The cell walls of cambial ray cells were thin; the thickness of radial walls was 0.77 µm and tangential walls, 0.21 µm (Table 2). Thickness of

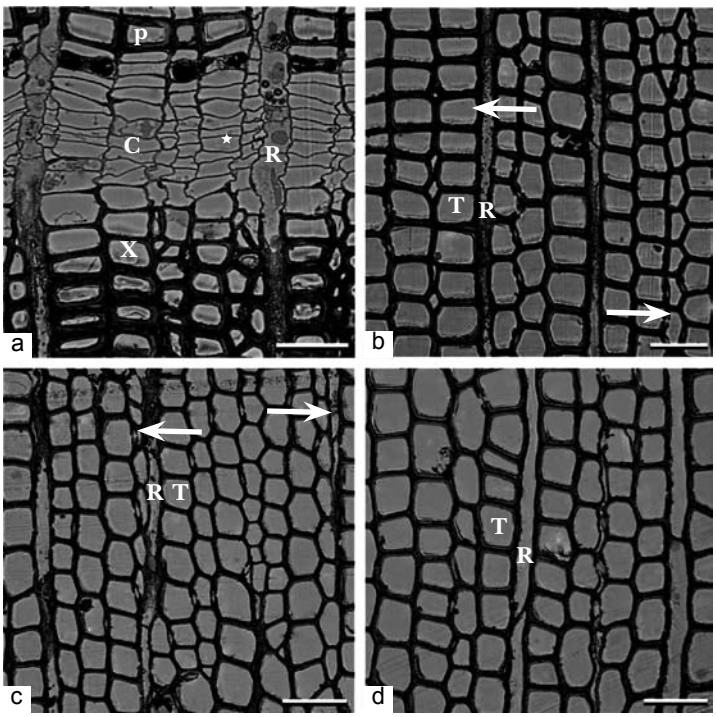

Figure 2 Light microscopy photographs of transverse sections of cells in *Cunninghamia lanceolata* showing microstructural changes from cambium to heartwood; (a) cambial cells showing thin cell wall, various shapes and radial enlarging (asterisk); (b) tracheids and ray parenchyma cells of middle sapwood demonstrating cell contents in ray parenchyma cells (top arrow) and hollow tracheary elements and bordered pits (lower arrow); (c) cell contents in ray parenchyma cells in intermediate wood (arrows); (d) ray parenchyma cells showing that protoplasm had disappeared in middle heartwood. All bars indicate 50 μm; P = phloem, C = cambium, X = xylem, T = tracheids, R = ray parenchyma cells.

Table 2 Changes in cell wall thickness of tracheids and ray parenchyma cells from cambium to heartwood

Cell wall	Cambium zone	Middle sapwood	Intermediate wood	Middle heartwood	Inner heartwood	F probability
Tracheids						
RW	0.76 a (0.31)	2.82 b (0.43)	3.41 c (0.49)	2.88 b (0.34)	2.79 b (0.29)	< 0.0001
TW	0.19 a (0.10)	3.67 b (0.67)	3.79 b (0.44)	3.36 c (0.32)	3.32 c (0.24)	< 0.0001
Ray parenchyma cells						
RW	0.77 a (0.23)	2.71 b (0.26)	2.54 b (0.15)	2.73 b (0.07)	2.71 b (0.12)	< 0.0001
TW	0.21 a (0.08)	1.53 b (0.17)	1.23 b (0.54)	1.30 b (0.08)	1.28 b (0.14)	0.0023

RW = radial wall, TW = tangential wall; values in parentheses represent standard deviations; $p < 0.05$; values with the same letter in the same row indicate no significant difference (Student Newman Keuls test).

the cambial ray cell walls, including radial walls and tangential walls, was significantly less than cell wall thickness of xylem ray cells ($p < 0.05$, Table 2). During their differentiation into xylem rays, the walls of the cambial ray cells became thick gradually (Figure 2a).

The cambial ray cells were rich with contents such as cell organelles and reserved materials, including starch and lipid droplets (Figures 3d, f, g). The vacuoles in ray cambial cells were small (Figure 3d) compared with cambial cells of fusiform cells.

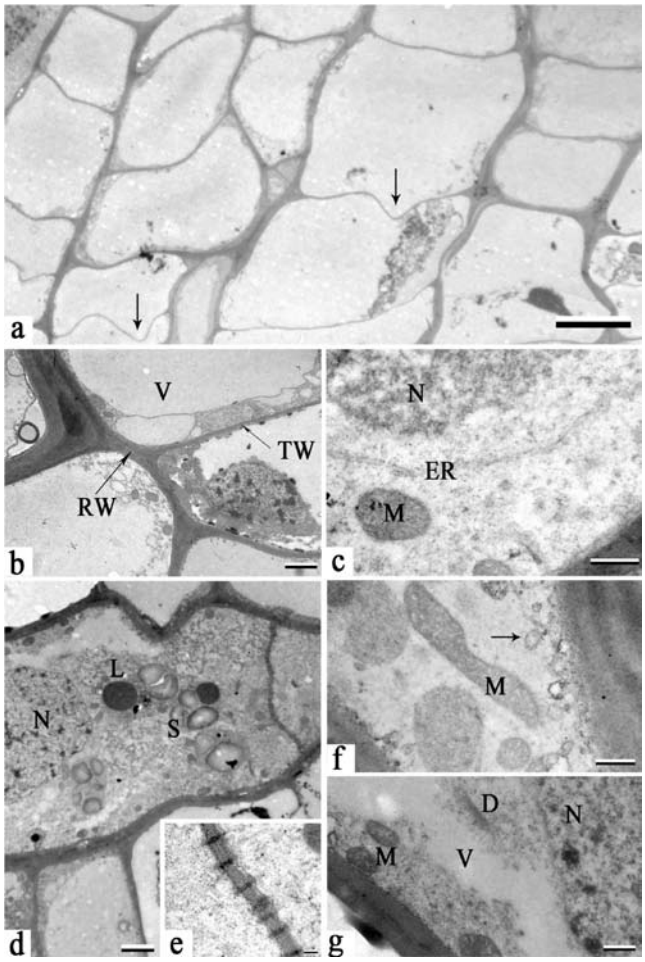

Figure 3 Transmission electron microscopy photographs of transverse sections of cells in *Cunninghamia lanceolata* showing the ultrastructure of cambial fusiform cells and ray cells in cambium; (a) highly vacuolated cambial fusiform cells showing thin cell walls, various shapes and the direction of radial enlarging (arrows); (b) cambial fusiform cells demonstrating large vacuoles (V), protoplasm around the periphery and thinner tangential wall (TW) than radial wall (RW); (c) nucleus (N), mitochondria (M) and endoplasmic reticulum (ER) in a cambial fusiform cell; (d and e) cambial ray cells showing lots of cell contents including starch (S), lipid droplets (L) and plasmodesma between two ray cells; (f and g) vacuole, nucleus, organelles, including mitochondria, endoplasmic reticulum and dictyosome (D), and vesicles (arrow) in cambial ray cells. Bars indicate 10 μm (a), 2 μm (b and d), 0.5 μm (c, f and g) or 0.2 μm (e).

Cellular changes of xylem tracheids and ray parenchyma cells

Radial wall thickness of xylem tracheids in the middle sapwood, intermediate wood, middle heartwood and inner heartwood reached 2.82, 3.41, 2.88 and 2.79 μm respectively, while the values for tangential wall at the same positions were 3.67, 3.79, 3.36 and 3.32 μm respectively (Table 2). No significant differences in thickness of radial walls were found between middle sapwood, middle heartwood and inner heartwood ($p < 0.05$), although thickness value of radial walls of intermediate wood was significantly higher than those of middle sapwood, middle heartwood and inner heartwood (Table 2). For tangential wall, significant differences were observed in thickness of the cell wall from intermediate wood to middle heartwood ($p < 0.05$). However, differences between middle sapwood and intermediate wood, and between the middle heartwood and inner heartwood were not significant (Table 2). The bordered pits between tracheids and hollow tracheids lumen were found in the middle sapwood (Figure 2b), intermediate wood (Figures 2c and 4e) and heartwood (Figure 2d).

The protoplasm of xylem tracheids was lost completely from middle sapwood to inner heartwood and became hollow dead tracheary elements (Figures 4a, e and i). The radial wall thickness of xylem ray parenchyma cells in middle sapwood, intermediate wood, middle heartwood and inner heartwood was 2.71, 2.54, 2.73 and 2.71 µm respectively. Thickness of the tangential walls at these positions reached 1.53, 1.23 1.30 and 1.28 µm respectively (Table 2). When compared with tracheids, the cell walls of ray parenchyma were thinner than those of tracheids adjacent to them from sapwood to heartwood (Figures 2b–d). No significant difference existed for cell wall thickness of xylem ray cells from middle sapwood to inner heartwood (Table 2). The cell wall structure of ray parenchyma showed typically polylamellate that was different from

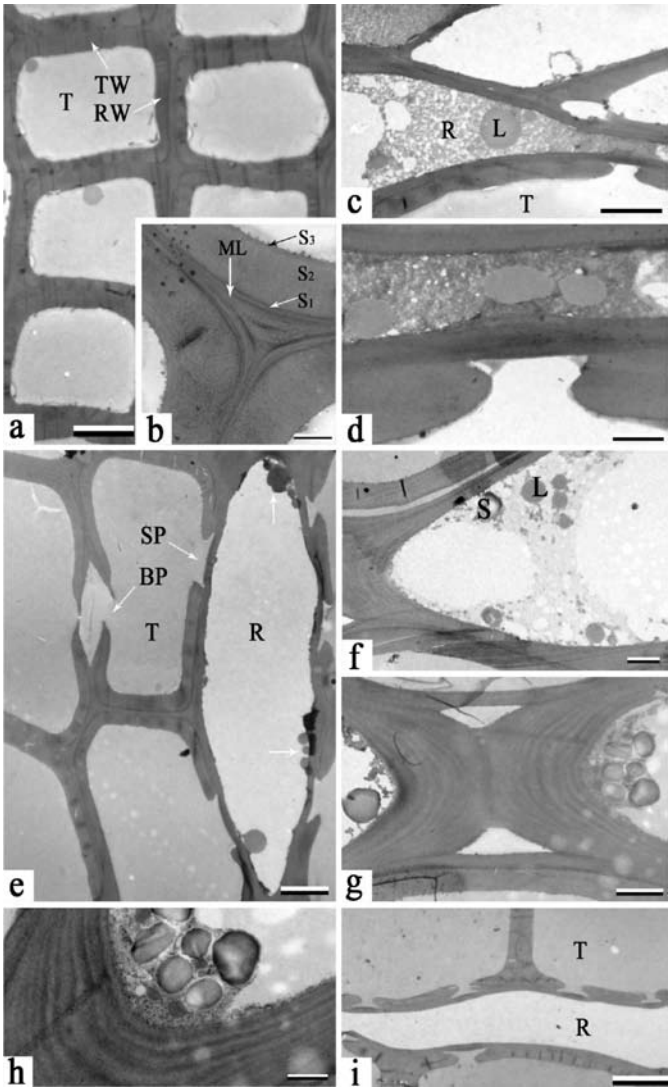

Figure 4 Transmission electron microscopy photographs of transverse sections of cells in *Cunninghamia lanceolata* showing the ultrastructure in middle sapwood, intermediate wood and middle heartwood; (a and b) tracheids (T) in middle sapwood showing lost protoplasm, thickened radial wall (RW) and tangential wall (TW), including S_1, S_2, S_3 and middle lamella (ML); (c and d) ray parenchyma cells (R) in middle sapwood demonstrating cell contents, including lipid droplets (L), and the semi-bordered pit between ray parenchyma and the tracheid; (e) tracheids and ray parenchyma cells in intermediate wood displaying the bordered pit (BP) between tracheids, semi-bordered pit (SP) between ray parenchyma cell and tracheids, and some cell contents (arrows) in ray parenchyma cell, (f, g and h) ray parenchyma cells in intermediate wood showing typically polylamellate structure and cell contents, including starch (S) and lipid droplets (L); (i) ray parenchyma cells in middle heartwood displaying the protoplasm had disappeared. Bars indicate 10 µm (a and i), 1 µm (b and h), 5 µm (c, d and e) or 2 µm (f and g).

the characteristically layered wall of the tracheid (Figures 4g and h). Plasmodesmatal connections were present in end walls between adjacent ray cells (Figure 3e). The semi-bordered pits between ray parenchyma cells and tracheids were visible (Figures 4c–e, i).

In comparison to protoplasm of cambial ray cells, the protoplasm of xylem ray cells, including reserved materials and organelles, became smaller and scattered in middle sapwood (Figure 2b and 4c) and intermediate wood (Figure 2c and 4f). The number of organelles, including mitochondria, endoplasmic reticulum and Golgi apparatus, declined from the cambial zone to intermediate wood (Table 3). Concomitantly, the shape of nuclei changed from spherical to elliptical then to spindle-shaped, and the nuclear slenderness ratio increased from 1.31 in cambial zone to 3.89 in intermediate wood (Figure 5a and b). For reserved materials, the amount of starch decreased in the direction from the cambium towards intermediate wood, but the amount of lipid droplets increased first from the cambium to middle sapwood then decreased towards the intermediate wood (Table 3). All ray parenchyma cells lost their protoplasm in the heartwood (Figures 2d and 4i).

The patterns of cellular changes of tracheids and ray parenchyma cells from cambium to heartwood are illustrated in Figure 6.

DISCUSSION

Cellular changes in cambial zone

Secondary vascular system consisting of cambial cells and their derivatives is an appropriate model system for understanding of *in situ*

Table 3 Changes of protoplasm characteristics in ray parenchyma cells from cambium to heartwood

Protoplasm characteristic	Cambium zone	Middle sapwood	Intermediate wood	Middle heartwood	Inner heartwood
Storage materials					
Starch	+++	++	+	-	-
Lipid droplets	++	+++	+	-	-
Organelle					
Mitochondria	+++	++	++	-	-
Endoplasmic reticulum	++	++	+	-	-
Golgi apparatus	++	+	+	-	-

+++ = abundant distribution, ++ = moderate distribution, + = infrequent distribution, - = no distribution

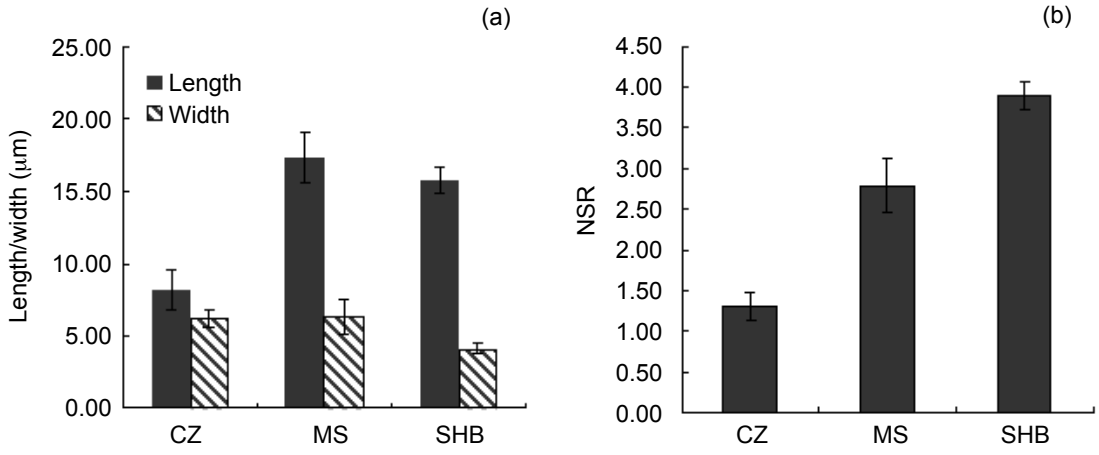

Figure 5 Changes of nuclear characteristics in ray cells at cambial zone (CZ), middle sapwood (MS) and intermediate wood (SHB) in *Cunninghamia lanceolata* during heartwood formation; (a) length and width of nuclei in ray cells, (b) nuclear slenderness ratio (NSR) of nuclei in ray cells

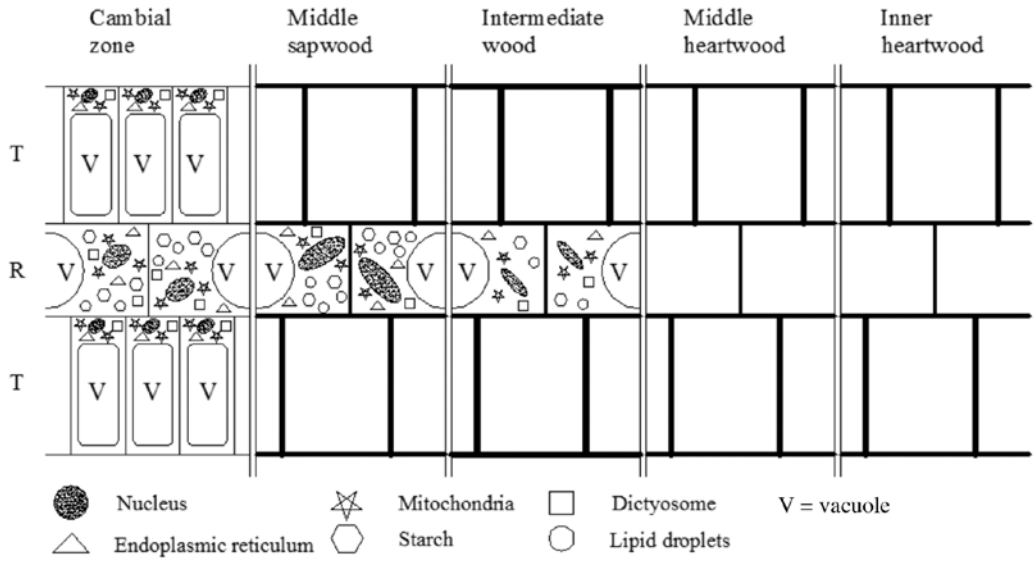

Figure 6 Schematic illustration showing the patterns of cellular changes in tracheids (T) and ray parenchyma (R) cells from cambium to heartwood

cytodifferentiation of wood tissues in trees (Nakaba et al. 2006, 2008). In this system, the differentiation from cambium cell to mature xylem in a radial direction could be followed and detected continuously.

During its life or existence, a wood cell undergoes significant morphological changes on its way from cambium to heartwood. Walls of fusiform cambial cells and ray cells were very thin and the thickness of radial walls was more than three times that of tangential walls, indicating differences in the deposition of the cell wall substances between radial walls and tangential walls. Radial expansion in cambial cells was also observed. This is due to the breakdown of acidic pectin network induced by radial wall lysis (Yin et al. 2002). Moreover, radial expansion is caused by the initial heterogeneous differences between radial walls and tangential walls; the former has more hemicellulose and pectin and less high molecular weight cellulose (Catesson & Roland 1981). The above two reasons increased the plasticity and extensity of radial walls, leading to the radial expansion in cambial cells.

Cambial fusiform cells were highly vacuolated and cytoplasm containing elliptical nuclei and lots of organelles was squeezed with a strip distribution around the periphery. Similar observations have been reported elsewhere (e.g. Wang & Cui 1998, Yin et al. 2002). Cells in the cambial zone had large vacuoles partly to resist the pressure from surrounding cells during their expansion and to meet the great demands of vigorous cambial activity. Increases in volume and water content in the vacuole cause the cell to enlarge in the cambial zone (Iqbal 1990).

Cambial ray cells were also highly vacuolated but their vacuoles were smaller compared with cambial fusiform cells. The cambial ray cells had high contents of organelles and reserved materials, including starch and lipid droplets, indicating vigorous activity in cambial zone.

Cellular changes in xylem zone

Secondary walls (S) of the tracheids in the middle sapwood, intermediate wood, and middle and inner heartwoods were deposited and displayed characteristically layered walls, which contained middle lamella, S_1 S_2 and S_3 layers (Figure 4b). In general, approximated cell wall thickness of tracheids was indicated from middle sapwood to inner heartwood, although the radial wall of tracheids was slightly thinner than tangential walls. The cell wall thickness from middle sapwood to inner heartwood was significantly greater than that of cambial fusiform cells.

In contrast, the cell wall in ray parenchyma cells showed typical polylamellate structure which was different from the characteristically layered wall of tracheids. This showed morphological and structural differences in the cell walls between tracheids and ray cells. Such structural features of ray parenchyma cell walls reflected a type of modified multi-net growth in which the orientation of microfibrils in the outer portion

of the wall becomes altered towards the vertical as cell elongation proceeds (Chafe & Wardrop 1972). For the cell wall thickness (including radial and tangential walls), there was no significance difference between xylem ray cells from middle sapwood to inner heartwood.

Tracheids of *C. lanceolata* lost their protoplasm during the thickening of secondary walls, and subsequently became hollow tracheary elements and declined into sapwood of xylem. Observation of cultured *Zinnia* cells showed that the onset of secondary wall thickenings preceded vacuole collapse by six hours, after which most of the cell contents vanished in several hours (Groover et al. 1997). Insulated hydrolytic enzymes were released when the vacuole collapsed, leading to the disintegration of organelles and final degradation of cell contents. It was also suggested that the vacuole and endoplasmic reticulum played a critical role in cell autolysis because of their acid phosphatase (Schulz & Jensen 1981). Ultrastructural studies of the degeneration processes in wheat nucellar cells showed that the autolysis of cytoplasm was caused by hydrolase coming from the Golgi apparatus (You 1985).

By contrast, ray parenchyma cells remained alive with protoplasm from the sapwood to intermediate wood (Figure 6). Research on *Acacia auriculiformis* indicated that nuclear slenderness ratio increased in ray cells contiguous to vessels but gradually decreased in ray cells away from vessels (Bhat & Patel 1982). However, Nair and Chavan (1983) showed that the nuclear slenderness ratios in *Acacia catechu*, *Bauhinia tomentosa*, *Bridelia retusa* and *Lagerstroemia speciosa* decreased gradually in the direction from the cambium towards the intermediate wood. In the present study, nuclear slenderness ratio of *C. lanceolata* increased from the cambial zone to intermediate wood. These results contradict findings from the former studies of hardwood, implying that the change in the shape of nuclei can be dependent on the species. After the nucleus changes its shape, it will disintegrate and chromatin will be lost with increasing distance from the cambium (Pandalai et al. 1985), indicating that activity and vitality of cells are gradually declining. The nuclei in ray parenchyma disappeared in middle heartwood and inner heartwood. Ray parenchyma could survive for at least 13 years, i.e. the growth time from cambium zone towards intermediate wood in *C. lanceolata*. The disappearance of nuclei was probably a signal of the death of ray parenchyma which marked the formation of heartwood.

There was a declining amount of starch grains in ray cells from the cambium to intermediate wood but the amount of lipid droplets increased first from the cambium to middle sapwood then decreased towards the intermediate wood, and finally all storage materials disappeared in the heartwood. This result was similar to the study on *Bridelia retusa* (Nair et al. 1981, Shah et al. 1981) but different from the research on *Tectona grandis* (Data & Kumar 1987). It has been suggested that the quantity and quality of DNA extracted from plastids (such as amyloplasts) decrease from outer sapwood to inner heartwood (Rachmayanti et al. 2009). Acid phosphatase is actively involved in the inter- and transcellular transport of carbohydrates and in hydrolytic processes in the cells (Matile 1975). Apparent acid phosphatase activity was observed in the middle sapwood which indicated the hydrolysis of starch grains and increased transport of carbohydrates (Nair et al. 1981). These carbohydrates may act as precursors for lipid biosynthesis, thus increasing lipid droplets in middle sapwood (Nair et al. 1981). Furthermore, during the heartwood formation, the depleted starch grains were observed in *C. lanceolata* and *Melia azedarach* (Pandalai et al. 1985) to show a granular periphery with lipid bodies of varying electron density associated with them. This showed that starch grains were broken down and mostly converted into lipid compounds. The absence of starch and lipids in the heartwood indicated that their degradation occurred during the transition from sapwood to heartwood. Starch and lipids play a prominent role in the maturation of ray parenchyma cells (Fukazawa et al. 1980) and biosynthesis of polyphenolic compounds (Hillis 1987) during the transition from sapwood to heartwood. Therefore, the structural and quantitative changes of reserve materials in ray parenchyma, including starch and lipids, may act as important factors in the pattern of differentiation and the process of heartwood formation. They are also better characteristics for identifying the boundary of sapwood and heartwood.

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