Fusiform cells in the cambium of Kalopanax pictus are exclusively mononucleate

Peter Kitin¹,², Yuzou Sano¹ and Ryo Funada¹,³

¹ Laboratory of Wood Biology, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
² Department of Dendrology, University of Forestry, Kliment Ohridski str. 10, Sofia 1756, Bulgaria

Received 22 June 2001; Accepted 28 September 2001

Abstract

While it is generally accepted that most plant cells are mononucleate, it has been argued with some vehemence that fusiform cambial cells can be multinucleate. The controversy has not been resolved since to date, studies by conventional microscopy and transmission electron microscopy have failed to confirm unambiguously whether cambial cells are mononucleate or multinucleate. In this study, semi-thin sections of epoxy-embedded specimens and thick slices of cambial tissues from the hardwood Kalopanax pictus were analysed by confocal laser scanning microscopy. Tangential sections of cambium, regardless of the thickness of the section, are likely to contain portions of cells in several adjacent layers of cells and, at the lower resolution of conventional microscopy, several adjacent cells can appear to be a single cell with more than one nucleus. The higher resolution in the third dimension of confocal microscopy allowed clearly adjacent layers of cells in the cambium to be distinguished and the number of nuclei per cell to be determined. In this tree, the cambial cells were mononucleate in all cases.

Key words: Cambium, confocal microscopy, Kalopanax pictus, nuclei.

Introduction

Cambial cells are cells whose specialized function is the production of elements of the secondary phloem and xylem. The structure and function of cambium have been reviewed frequently and in depth (Philipson et al., 1971; Barnett, 1981; Catesson, 1990, 1994; Iqbal, 1990; Larson, 1994; Chaffey, 1999; Lachaud et al., 1999). It has been established that the dimensions and the morphology of the nuclei in cambial cells, as well as the number of nucleoli, vary within a single species according to both the season and the stage of cambial activity (Barlow, 1985; Mellerowicz et al., 1989, 1990, 1992; Lloyd et al., 1994, 1996).

It is generally accepted that each cambial cell contains a single nucleus (Bailey, 1920; Larson, 1994). However, for the past 100 years, some botanists have argued that large and extremely elongated plant cells, such as cambial cells, might contain many nuclei (for references, see Bailey, 1920). In addition, during the past 30 years there have been reports that the fusiform cambial cells in some tropical species are multinucleate (Ghouse and Khan, 1977; Iqbal and Ghouse, 1987; Venugopal and Krishnamurthy, 1989; Iqbal, 1990; also, for references, see Larson, 1994). Seasonal variations in the number of nuclei have also been reported and, in some species, cells containing five nuclei (Ghouse and Khan, 1977) or eight to ten nuclei (Iqbal and Ghouse, 1987) have been described. However, the existence of multinucleate cambial cells has not been established unambiguously. It has been suggested that a cambial cell might appear erroneously to be multinucleate under the microscope if two or more cells are superimposed in a tangential section (for discussion, see Larson, 1994; Farrar and Evert, 1997). Nevertheless, it has been noted that, if multinucleate cambial cells do indeed exist, they represent a curious phenomenon that necessitates detailed studies of the division of such cells, providing new avenues of research for cambial cytologists (Iqbal and Ghouse, 1990; Catesson, 1994; Lachaud et al., 1999). The number of nuclei in cambial cells has been inadequately studied and it remains to be clearly determined whether cambial cells are mononucleate or multinucleate.

This study was designed to analyse the nuclear status of fusiform cambial cells in the hardwood Kalopanax
pictus Nakai (Araliaceae). Conventional microscopic examinations of 15 μm-thick tangential sections of cambium revealed cambial cells that appeared to have multiple nuclei (P. Kitin and R. Funada, unpublished observations). By contrast, no multinucleate cells were detected among isolated cambial cells in macerated tissue (Kitin et al., 1999) or in single cells after three-dimensional (3-D) reconstruction using micrographs of serial sections of epoxy-embedded tissue (Kitin et al., 2000). In the present study, it was attempted, by serial optical sectioning with a confocal laser scanning microscope, to determine the number of the nuclei in individual cells. The confocal microscope is an appropriate tool with which to study large intact cells in thick slices of tissue and allows detailed analysis of the nuclear status of cambial cells (Kitin et al., 2000; Funada, 2001).

**Materials and methods**

**Plant material**

Small blocks of tissue, including cambium and the adjacent phloem and xylem, were cut with a sharp knife and a chisel from the stem of a single specimen of *K. pictus* that was growing on the campus of Hokkaido University. Blocks were fixed in FAA or glutaraldehyde solution, as described previously (Kitin et al., 1999). Samples were obtained in late autumn (5 November) and in spring (3 April, 22 April and 1 May).

**Preparation of samples and microscopy**

Samples of cambium were examined by conventional microscopy and by confocal laser scanning microscopy (CLSM). The samples examined included tangential and radial sections (15–20 μm thick) of celloidin-embedded specimens, serial semi-thin tangential sections (1–5 μm thick) of epoxy-embedded specimens, and thick tangential sections (60–80 μm thick), as described previously (Kitin et al., 1999, 2000). All specimens were stained with a 1% solution of safranin in 30% ethanol for 10 min under a vacuum and then for 1 h in an incubator at 35 °C (Kitin et al., 2000).

The 1–5 μm-thick and 15–20 μm-thick sections were mounted in mounting medium (Bioleit; Oken-shoji, Tokyo, Japan) for preparation of permanent microscope slides. The slides were observed with a confocal laser scanning microscope (LSM-310; Carl Zeiss, Oberkochen, Germany) under transmitted visible light or after excitation by incident light from an argon ion laser (wavelength, 488 nm) with a band-pass (BP) filter (515–565 nm) or from a helium neon laser (wavelength, 543 nm) with a long-pass (LP) filter (590 nm).

The sections of 60–80 μm thickness were treated to remove excess stain from cells. They were dehydrated through a graded acetone series (30%, 50%, 75%, 90%, and 100%; 15–30 min at each concentration), with frequent changes of the respective solutions of acetone, until no further colour was extracted from the specimens. Then the specimens were rehydrated by passage through decreasing concentrations of acetone and finally they were placed in distilled water. Next, the specimens were passed through increasing concentrations of glycerol (25%, 50%, 75%, 100% for 1 h or more per solution), with two or three changes of each solution, and left overnight in 100% glycerol. They were mounted on glass slides in glycerol and coverslips were placed on the samples. Incident light from a helium neon laser (wavelength, 543 nm; LP filter, 590 nm) was used for excitation for observations of the 60–80 μm-thick sections of cambium by CLSM. Consecutive confocal images of tangential sections of cambium were obtained at intervals of 1, 2 or 3 μm. Either a Plan Neofluar 40 × 0.75 air or a Plan Neofluar 63 × 1.25 oil objective lens (both from Carl Zeiss) were used.

The digital confocal images were stored on a computer and printed with a digital colour printer (UP-D8800; Sony, Tokyo, Japan) as described previously (Furusawa et al., 1998). Entire cambial cells were analysed to determine their nuclear status on images of serial optical sections on a computer using the software of the confocal laser scanning microscope.

**Results and discussion**

Cambial cells that appeared to have several nuclei were detected in the 15–20 μm-thick tangential sections in all samples of dormant (5 November) and active (3 April, 22 April, 1 May) cambia of *K. pictus* (Fig. 1A, B). However, multinucleate fusiform cambial cells on tangential sections must be examined carefully to determine whether the cells are, indeed, single cells or actually represent superimposed cells in adjacent layers of cells. Under the conventional light microscope, it was not easy to distinguish adjacent layers of cells in tangential sections of more than 10 μm in thickness because the tangential walls of adjacent cells were visible only with difficulty (Fig. 1A). Observations of sections of 1–5 μm in thickness did, however, distinguish cells in adjacent tangential layers (Kitin et al., 2000). The tangential walls of cambial cells could be observed with relative ease when the sections were slightly oblique (note, for example, the obliquely cut double walls between cells 1, 2 and 3 in Fig. 2). In contrast to the observations of 15–20 μm-thick sections, observations of 1–5 μm-thick sections failed to reveal any multinucleate cells. When nuclei of adjacent cells were visualized in the same focal plane, it was apparent that such nuclei were positioned on both sides of obliquely cut cell walls (Fig. 2). Similarly, in studies by transmission electron microscopy (TEM), no multinucleate cambial cells were found in *Robinia pseudoacacia* (Farrar and Evert, 1997) or in *Aesculus hippocastanum* (Chaffey et al., 1997). However, it is difficult to analyse entire cells on single sections of 1–5 μm in thickness or by TEM using ultra-thin sections and a clear definition of the nuclear status of cambial cells cannot be derived in either case.

Entire cambial cells can be analysed in detail on serial sections of epoxy-embedded tissue. However, the procedure is very time-consuming (Kitin et al., 2000). In the present study, the nuclear status of cambial cells was analysed on serial optical sections of thick slices of tissue (60–80 μm thick) by CLSM. Multinucleate cambial cells were visualized in projection images by CLSM (Fig. 3A). Three-dimensional (3-D) analysis of the same slices of tissue did not, however, show any cambial cells with...
more than one nucleus. The higher resolution of the confocal images in the third dimension ('z'-resolution) revealed the tangential walls between adjacent cells and showed that each nucleus did, in fact, belong to a different cell (Fig. 3B, C, D). The bright strip between cells 1 and 2 in Fig. 3B, C and D is due to fluorescence from the obliquely cut tangential walls between these two cells. The nucleus of cell 1 is seen in panels B and C in Fig. 3 and the nucleus of cell 2 is seen in panel D in the same figure.

The number of nuclei in cambial cells has been studied, for the most part, in tangential sections by conventional light microscopy. Iqbal and Ghouse reported as many as 10 nuclei per cell in their analysis of 10–12 μm-thick longitudinal tangential sections of the cambium of Acacia nilotica (Iqbal and Ghouse, 1987, 1990). These authors argued that a 10–12 μm-thick tangential section would contain, at most, two or three layers of cambial cells since the radial diameter of fusiform cambial cells was only 4–10 μm. Therefore, the detection of, apparently, 10 nuclei per cell in 10–12 μm-thick sections was taken as proof of the existence of multinucleate cells.

In the present study, cambial cells that were apparently multinucleate were also observed in relatively thin sections by conventional microscopy or in projection images obtained by CLSM. For example, Fig. 1B is a projection image that was constructed from ten optical sections obtained, at steps of 1 μm, by CLSM. The tissue scanned by CLSM corresponded, in this case, to a histological section of 9 μm in thickness. The image in Fig. 1B clearly shows the fluorescence from several nuclei
per cell, which are included within the 9 μm-thick optical slice of cambial tissue. Since the radial width of fusiform cambial cells in the stem of *K. pictus* is 6–12 μm, a tangential section of 9 μm would normally contain portions of at most one or two layers of cells. Therefore, the visualization of four nuclei within a tangential section of 9 μm in thickness suggests the presence of cells with more than one nucleus or, at least, of two superimposed cells that are in the process of mitosis, provided that the section is oriented exactly along the cell axes. However, the analysis by CLSM revealed that, in every case, the tangential sections of cambium were slightly oblique.

Even thin sections, such as the section shown in Fig. 2, included portions of several adjacent cambial cells. Figure 4 shows that it is extremely difficult and may even be impossible to cut longitudinal sections precisely along the axes of cambial cells. Tangential sections for observations of cambium are commonly large (side AB in Fig. 4). Thus, a very small shift in the plane of sectioning from the axial direction (the angle α in Fig. 4) would result in non-negligible obliqueness of the section. Since the length of the fusiform cambial cells in *K. pictus* can be as much as 600 μm (Kitin et al., 1999), the longitudinal extent of any observation on a microscopic

---

**Fig. 3.** A confocal image of a thick tangential section of cambium (thickness, approximately 60 μm) that was sampled at cambial reactivation (3 April). The section was stained with safranin and excited by the helium neon laser (543 nm) with an LP filter (590 nm). The images show the bright fluorescence from cell walls and nucleoli. (A) A projection image of eight optical sections, obtained in 2 μm steps. Two nuclei appear within the contours of some of the fusiform cells (arrows). However, an analysis of confocal images shows that each of the nuclei belongs to a different cell. (B–D) Sequential optical sections in 2 μm steps of the sample in (A). Cells designated 1 and 2 in (B–D) are adjacent cells in a radial file of cambial fusiform cells. The bright strip between cells 1 and 2 is a portion of the tangential wall between these two cells, which was cut obliquely. The nucleus of cell 1 is seen in panels (B) and (C) and the nucleus of cell 2 is seen in panel (D). Scale bar = 50 μm.
Acacia nilotica (L.) Del. var. toli a Troup (Mimosaceae) in 1934. Seasonal variation in the nuclear number of fusiform cambial initials in Robinia pseudoacacia. Trees 11, 203–215.


Ghouse AKM, Khan MIH. 1977. Seasonal variation in the nuclear number of fusiform cambial initials in Psidium guajava L. Caryologia 30, 441–444.

Hejnowicz Z, Romberger JA. 1979. The common basis of wood grain figures is the systematically changing orientation of cambial fusiform cells. Wood Science and Technology 13, 89–96.


Iqbal M, Ghouse AKM. 1987. Anatomy of the vascular cambium in Acacia nilotica (L.) Del. var. telia Troup (Mimosaceae) in treatment with the conventional light microscope. The results of this study demonstrate that portions of several adjacent fusiform cells are likely to be superimposed in tangential sections, appearing to be a single cell with several nuclei, because of the large depth of focus and low resolution in the third dimension of the conventional light microscope. By contrast, analysis by CLSM of serial optical sections of thick slices of tissue clearly revealed that each cambial cell of K. pictus contains a single nucleus.

Acknowledgements

The authors thank Dr J Ohtani and Dr S Fujikawa for their valuable comments. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. JSPS-RFTF 96L00605).

References


Ghouse AKM, Khan MIH. 1977. Seasonal variation in the nuclear number of fusiform cambial initials in Psidium guajava L. Caryologia 30, 441–444.

Hejnowicz Z, Romberger JA. 1979. The common basis of wood grain figures is the systematically changing orientation of cambial fusiform cells. Wood Science and Technology 13, 89–96.


Iqbal M, Ghouse AKM. 1987. Anatomy of the vascular cambium in Acacia nilotica (L.) Del. var. telia Troup (Mimosaceae) in...


