THE SEASONAL OCCURRENCE AND HISTOLOGY OF SEPTATE FIBERS IN KALOPANAX PICTUS

by

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SUMMARY

Septate wood fibers were abundant in the following parts of growth rings of Kalopanax pictus Nakai: 1) around the vessels, 2) in the vicinity of ray cells, 3) in terminal regions of the growth rings. Septum formation in wood fibers progressed from the initial region (pore zone) towards the terminal region within a current growth ring with progressing lignification of the wood fiber walls. Many septate wood fibers at the end of the growth ring had radially continuous septa. Karyokinesis was observed in several wood fibers before the initiation of septum formation, while lignification was in progress after the completion of the S₃ layer deposition. This suggests that the septation starts in parallel with the progress of lignification after the deposition of the S₃ layer.

Key words: Kalopanax pictus Nakai, lignification, septate wood fiber, septation.

INTRODUCTION

A septate wood fiber is defined as having thin transverse walls across the lumen (IWA 1964). The existence or occurrence of septate wood fibers in xylem is referred to as a discriminative feature in dicotyledonous wood anatomy (Butterfield & Meylan 1976; Archer & Van Wyk 1993; Dong Zhongmin & Baas 1993; Li Baizhong et al. 1995). In general, wood fibers which mainly act as mechanical support in the xylem have been thought to be dead cells which lost their cytoplasmic contents. In many species, however, the existence of living wood fibers, which contain nuclei and cytoplasm in their lumina even after completion of lignification of the cell walls, has been reported (Bailey 1953; Fahn & Leshen 1962; Fahn & Arnon 1962; Chalk 1983; Murphy & Alvin 1997). Septate wood fibers also are considered as living fibers in which nuclei, cytoplasm, and storage substances are present in the cell lumen (Vestal & Vestal 1940; Harrar 1946; Spackman & Swamy 1949; Govindarajaru & Swamy 1955; Chowdhury et al. 1956; Ghosh & Purkayastha 1960). In certain species the septate wood fibers contain crystals (Chattaway 1955; Purkayastha 1958).

Septate wood fibers closely resemble axial parenchyma cells in distribution or arrangement within a growth ring, and seem to substitute for them as regards function (Harrar 1946; Spackman & Swamy 1949; Ghosh & Purkayastha 1960). Septate wood
fibers commonly were distributed in both apotracheal and paratracheal patterns in a survey of 110 dicotyledonous families by Spackman and Swamy (1949). They noted that the higher the number of septate wood fibers, the lower the number of xylem parenchyma cells (Spackman & Swamy 1949). Presumably, septate fibers are thus performing the dual functions of storage and mechanical support (Govindarajaru & Swamy 1955; Chowdhury et al. 1956). Although the storage and mobilization functions of parenchyma cells have been well studied (Zimmermann 1971), those of septate wood fibers have not. The relationship of septate wood fibers to vessels and to parenchyma cells should be investigated to clarify possible functions.

Vestal and Vestal (1940) observed the ontogeny of septa in fiber tracheids of *Hypericum androsaemum* L., and showed that the karyokinesis of fiber tracheids is followed by cytokinesis. Close observations of septum formation in wood fibers of *Lagerstroemia speciosa* L. and *Protium serratum* Wall. ex Colebr. by Purkayastha (1958) showed that the septum formation occurs after the completion of cell elongation and secondary wall deposition. Parameswaran and Liese (1969) observed seasonal development of septate wood fibers accompanied by cytological changes in shoots of *Ribes sanguineum* Pursh using transmission electron microscopy, and showed changes in organelle quantity and localization before and after septation. The septum in wood fibers seems to be formed during a single growing season in *H. androsaemum* (Vestal & Vestal 1940). It was reported that about two weeks in *L. speciosa* and about four weeks in *P. serratum* are required for the formation of septa, after the wood fibers became distinguishable from the cambium (Purkayastha 1958).

There is little histological information on how these wood fibers occur in the xylem. To understand the formation of septa, it is necessary to observe both the development and maturation of the fibers. In this study, *Kalopanax pictus* is used to study the distribution of septate wood fibers within a single growth ring, and to relate septal development with fiber wall ontogeny.

**MATERIALS AND METHODS**

Seven straight young *Kalopanax pictus* trees growing in the Nikko Experimental Forest of Utsunomiya University, Japan, were harvested from early July to mid October every two weeks (July 10, 25, August 10, 26, September 11, 26, October 9, 1996). Some disks were cut from the sample trees at a height of 1.2 m. Small blocks including the cambial zone and the latest growth ring boundary were prepared from the disks, and were fixed in FAA. Radial sections of 10 μm in thickness were cut, stained with safranin, and used to observe the occurrence of septate wood fibers within growth rings. The process of secondary wall formation of wood fibers was observed using cross sections under a polarized light microscope. The lignification process in the secondary walls was observed using transverse sections stained with Wiesner and Mäule color reagents. The chemical components of the septum were examined by using some staining regents (safranin, light green, aniline blue, methylene blue, chlorzinciodine solution, ruthenium red, Mäule and Wiesner color reagents). Starch grains in septate wood fibers were stained with I2-KI reagents.
Fig. 1. Occurrence of septate fibers in *Kalopanax pictus*. – a: Pore zone (region 1); radial section. – b: Septate fibers neighboring ray cells (region 2); tangential section. – c: Septate fibers neighboring latewood vessels (region 1); radial section. – d: Terminal region (region 3). Septa line up horizontally in radial sectional view. — Arrowheads = septum; AP = axial parenchyma cell; V = vessel; R = ray parenchyma cell; GRB = growth ring boundary. — Scale bars = 30 μm.
Small wood strips for maceration were obtained from an 8-year-old tree. After being fixed in FAA, the samples were divided into five segments radially from initial to terminal regions in growth rings, and macerated with Jeffrey’s reagent to observe the frequency of occurrence of septate wood fibers within growth rings. Frequency was determined as the number of septate wood fibers per 100 wood fibers.

RESULTS AND DISCUSSION

Functional distribution of septate wood fibers

The xylem structure of *Kalopanax pictus* has been described by Sudo (1959), Ishida and Ohtani (1989), and Simaji and Itoh (1992). *Kalopanax pictus* is a typical ring-porous wood (with a single pore zone) with latewood vessels in tangential wavy arrangement.

Septate fibers with one septum each were distributed in the following regions within a growth ring, as shown in Figure 1: 1) around the vessels (both wide and narrow ones), 2) in the vicinity of ray cells, 3) in terminal regions of the growth rings. A cross-sectional view did not show any parenchyma-like bands or arrangements as reported earlier (Spackman & Swamy 1949; Ghosh & Purkayastha 1960). In *K. pictus* some septate fibers were present with vasicentric parenchyma cells around large vessels in the pore zone and around small latewood vessels (Fig. 1a, c). These septate fibers possessed wider lumina than those localized in the terminal regions. In *K. pictus*, the percentage of axial parenchyma cells in the xylem is very small, 2% (Saiki & Harada 1994), but most parenchyma cells occur around wide vessels in the earlywood. Septate wood fibers are localized in the vicinity of ray cells throughout growth rings (Fig. 1b). If septate fibers function as axial parenchyma cells, then those localized in the regions 1) and 2) may function in transport and storage, as pointed out by several investigators (Harrar 1946; Govindaraju & Swamy 1955; Chowdhury et al. 1956; Buvat 1989). Alternatively, one could state that fibers localized in these regions may form septa preferentially to substitute for the functions of parenchyma cells, because of the small number of axial parenchyma cells in *K. pictus*. Imagawa (1984) mentioned in his study of the xylem development in *K. pictus* that the secondary wall formation and lignification of the cells associated with earlywood vessels progresses faster than those in other cells. The maturation rate of vessel-associated wood fibers (Region 1) may be related to septation.

Figure 2 shows frequency of occurrence of septate wood fibers within a growth ring. The first half part of the growth ring showed low frequencies (Fig. 2: F1–F3). Thereafter, it largely increased towards the terminal region of each growth ring (Fig. 2: F4–F5). This specific occurrence pattern within a growth ring corresponded with the observation using radial sections.

Septate wood fibers with dense starch grains were abundant in the the terminal region (Fig. 2: F5). This was observed throughout the sapwood. Radial sections showed that septate wood fibers frequently have horizontally aligned septa in this region as shown in Fig. 1d. It is considered that such an arrangement of septa is a result of the symplastic transverse division of these wood fibers.
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Fig. 2. Frequency distribution of septate fibers within one growth ring in sapwood and heartwood.

**Histochemistry of septa**

Parameswaran and Liese (1969) histochemically demonstrated the presence of cellulose and pectin, and the absence of lignin in the septa of *Ribes sanguineum*. In contrast, there are some reports that septa are lignified in *Lagerstroemia speciosa* (Purkayastha 1958) and in *Oxytenanthera abyssinica* (Parameswaran & Liese 1977). In our material of *K. pictus* septa were well stained with light green, aniline blue, methylene blue, and chlorziniodine solutions, as well as ruthenium red. However, neither Mäule nor Wiesner reagents stained the septa. This indicates that the septa are composed of cellulosic materials and pectin, but not of lignin, just as reported for *Ribes* sp. (Parameswaran & Liese 1969).

**Development of septa**

The septum is a secondary structure which is formed after the maturation of the wood fiber (Parameswaran & Liese 1969; Butterfield & Meylan 1976; Ohtani 1987). There are only few reports on the time required for septation after wood fibers have formed from the cambium. The time required for the septation varies with the species, the growth environment, and even within an individual depending on the stage of fiber development. *Lagerstroemia speciosa* seems to require about two weeks for septation, whereas *Protium serratum* requires four (Purkayastha 1958). However, the detailed relation between wood fiber development and septation has not yet been investigated in many species.
Fig. 3. The radial section of the sample harvested on July 10. No septa were observed in the lignified wood fibers (large arrowheads) located in the pore zone. — AP = axial parenchyma cells (small arrowheads). — Scale bar = 30 μm.

In *K. pictus*, collected on July 10, almost all of the wood fibers were in the enlarging developmental stage, except for those in the pore zone, the secondary walls of which had already completed the deposition of the S₃ layer and lignification. However, septa were absent in these wood fibers of the pore zone (Fig. 3). In the sample collected on July 25, lignification of secondary walls in wood fibers formed outside the pore zone was progressing towards the cambium, no septa being formed. In this sample, the occurrence of septa was restricted to the pore zone. In the sample collected on August 10, the number of septa gradually increased towards the cambium. In the sample of September 26, septa were present diffusely in the entire xylem formed in the current year except for the terminal region, although septa were seen in the vicinity of small vessels. In the sample of October 9, many septa were also present in the terminal region (Fig. 4). These results show that septum formation in wood fibers progresses from the initial region towards the terminal region within a current growth ring accompanied by the progress of lignification in wood fiber walls. This agrees with data on *Lagerstroemia speciosa* and *Protium serratum* (Purkayastha 1958), and *Ribes sanguineum* (Parameswaran & Liese 1969). On the other hand, karyokinesis was not found in fibers of the earlier growth rings. This indicates that the formation of septa is completed within one growing season.
The samples collected on September 11, 26, and October 9 were compared to clarify the relationship between wood fiber development and the occurrence of septa. A model of the developmental process of wood fiber walls and septum is illustrated in Figure 5. A cross section prepared from the sample of September 11 shows that the cambial activity had almost ceased (Fig. 5a1). The formation of septa was not observed up to about the fifteenth wood fiber from the cambium in the radial section. The S₂ layer was already deposited or was being deposited in some wood fibers near the cambium. Deposition of an S₃ layer was not observed in the fifth to seventh wood fibers from the cambium (Fig. 5a1 & a2). In several wood fibers where deposition of an S₃ layer had already been completed, it was observed that karyokinesis was in progress accompanied by lignification; the wood fibers likely being at a stage just before septum formation. In the sample of September 26, the number of septate wood fibers increased with the continued maturation of the wood fibers when compared to the situation of September 11 (Fig. 5b1 & b2). Wood fibers without septa were limited to several layers (about up to the fifth cells) near the cambium. The deposition of the S₃ layer was found in the outermost wood fibers near the cambium except for the terminal wood fibers, indicating that the secondary wall formation of wood fibers had almost been completed. These wood fibers were more strongly lignified than those in the same region in the sample of September 11. The sample of October 9 showed that wood fibers localized in the terminal region had almost completed cell wall forma-
Fig. 5. A model of the development of wood fiber walls and septa. – a1, b1, c1: Polarized light microphotographs of cross sections. White arrowheads = outermost wood fiber with an $S_3$ layer. – a2, b2, c2: Diagrams illustrating development of secondary wall layers in radial longitudinal section. Arrows show the completion of each stage of wood fiber wall development. $S_2$, $S_3 = S_2$ or $S_3$ layer deposition; $L =$ completion of lignification; SF = septum formation. — Scale bars = 20 μm.
tion. In addition, septum formation was also found in wood fibers near the cambium (Fig. 5c1 & c2). This corresponds with the results shown in Figure 4: septum formation is accompanied by cell wall maturation of wood fibers and is commenced after the completion of lignification. Figure 6 shows the presence of nuclei in wood fibers in the late telophase of cell division and the occurrence of a phragmoplast between the daughter nuclei. This feature seems to be an indicator of the final stage of septation (Vestal & Vestal 1940). These events were observed in the wood fibers undergoing lignification. It is considered that septation occurs simultaneously with lignification after the completion of secondary wall deposition.

It has been previously thought that septum formation occurs after secondary wall formation. This hypothesis was confirmed by Purkayastha (1958) using Lagerstroemia speciosa. The same is true for Kalopanax pictus. However, the septation in wood fibers should be considered relative to lignification. Septate wood fibers retain living cytoplasm for a long time. In K. pictus, septum formation in wood fibers apparently occurs after the completion of lignification in their cell walls. This study shows that the septation, including karyokinesis, cytokinesis and septum formation, occurs in parallel with the progress of lignification after the deposition of S₃ layer: karyokinesis first occurs in wood fibers, followed by cytokinesis, and then septum formation is commenced just after the completion of lignification.

REFERENCES

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