Developmental anatomy of the fiber in *Phyllostachys edulis* culm

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With several methods of microscopy, the differentiation and development of fibers in the middle third of *Phyllostachys edulis* culm walls were systematically studied. The development of fibers could be divided into three stages: the formation of fiber initials, primary wall and secondary wall. Fibers originated from the same procambium as the vascular bundle elements like vessels and sieve tubes, but their differentiation is later. Fibers centrifugally underwent differentiation and development outwards from a vascular bundle. During primary wall formation, most fibers were bi-nucleate or multi-nucleate contributing to their elongation, which might be related to amitosis. During secondary wall formation, fiber wall underwent dominant thickening during the first 4 years, and then the degree of wall thickening decreased gradually. With the thickening of secondary wall, fiber nucleus persisted for many years in the culms investigated. The plasmodesmata and transfer vesicles were also persistent in the pits between the fibers and their adjacent cells. The results demonstrated that the fiber in the middle third of *Phyllostachys edulis* culm walls is a kind of long-living cell. The persistence of fiber nucleus and plasmodesmata and transfer vesicles is closely related to the thickening of secondary wall with aging.

*Phyllostachys edulis* (Carr.) H. De Lehaie has the largest distribution area and the highest economical value in China. Because of the high fiber content of 38% (Grosser & Liese, 1974), it is widely used for making furniture, construction, pulp and other industries in China. The variation in the structure and properties of fibers with aging has a decisive impact on the property of bamboo culms, and in turn, dramatically affect culm utilization. The morphology, chemical components and tissue ratio of fibers in *Phyllostachys edulis* culm were studied by Parameswarn and Liese (1976) and Xiong et al. (1980b). Some anatomical changes during the development of fibers were also reported (Xiong et al., 1980a; Liese and Weiner, 1997; Murphy and Alvin, 1997; He et al., 2000). Little is known about systematic anatomical studies of fiber development so far.

In this paper, further studies of the origin and developmental anatomy, as well as the long-living character of fiber in *Phyllostachys edulis* culms were reported in detail. Due to the difference of the position of the vascular bundles and the location of the fibers within, the developmental pattern among fibers within, the developmental pattern among fibers across a culm wall is different (Liese, 1998). Only the fibers in the middle third of the culm wall were investigated in this work.

**MATERIALS AND METHODS**

**Materials**

Young shoots and culms of 1 to 16 year old *Phyllostachys edulis* were harvested in April 2001 at the Bamboo Garden and the experimental forestry farm of Nanjing Forestry University. Young shoots with heights of 60 cm, 80 cm, 120 cm and 700 cm were collected, and samples about one-cm³ from the middle third of the culm wall in the middle part of every internode of the young shoots taken. For 1 to 16-year-old culms blocks about one-cm³
were sampled only from the middle third of culm wall in the middle part of internodes in the middle part of the culms. (Fig. 1)

Methods

Preparation for light microscope

The blocks from young shoots were immediately fixed in FAA (formalin, acetic acid and ethyl alcohol) with 50% alcohol, and the blocks from 1 to 16-year-old culms in FAA with 70% alcohol. Transverse and longitudinal sections of young shoots were made with a routine paraffin method and stained with safranin-fast green. For the 1 to 16 years old culms, sections were made with GMA (glycol methacrylate) or PEG (polyethylenglycol) 2000 method, and stained with safranin-alstrablue, crystal violet, or acridin orange. All sections were examined and photographed with a biological optical microscope (OLYMPUS, Japan).

Preparation for scanning electron microscope

Blocks of approximately one-cm³ from 1 to 16-year-old culms were treated with FAA, and cooked at 1.2 bars in an autoclave for 4 hours. Later the surface of the transverse samples was cleaned with a sharp blade. Following dehydration with serial gradient alcohol from 50 to 100%, the samples were dried with the critical point method. All samples were examined and photographed with a JSM-6300 scanning electron microscope.

Preparation for transmission electron microscope

Blocks of approximately one-mm³ from 1 to 16-year-old culms were fixed in 2.5% glutaraldehyde (in 0.025 mol/l phosphate buffer, pH7.0) for 4 hours. After washing with the same buffer, the samples were post-fixed in 1% OsO₄ (also in the same buffer) for 3 hours. Followed by a further washing with the buffer, the specimens were dehydrated in a graded ethanol series and embedded in Spurr’s resin. After cutting with a diamond knife on a LKB-V ultramicrotome, ultrathin sections were stained with saturated aqueous uranyl acetate for 5 min, followed by 5 min in lead citrate. Finally all sections were examined and photographed with a H-600 transmission electron microscope (TEM).

RESULTS

The successive development of bamboo fibers could be divided into three stages: formation of fiber initials, primary wall and secondary wall.

Fiber initials formation

The terminal meristem in longitudinal sections consists of tunica and corpus from where the primary meristem was derived (Fig. 2, a). With further development and differentiation, some cells elongated in axial direction forming procambium(Fig.2, b). These cells were much
Figure 2. a: the structure of shoot apex, showing tunica, corpus, LS; b: the procambium with diffuse chromatin, LS; c: the early procambium strands with four cells (arrowheads), TS; d: procambium strands with a cluster of cells (arrowheads), TS; e: the formation of prophloem sieve tube (arrowheads), TS; f: the formation of protoxylem vessel (arrowheads), TS; g: the formation of fiber initials (arrowheads), TS; h: the fibers during co-development with vascular bundle, showing bi-nuclei or multi-nuclei phenomenon(arrowheads), LS; i: the fibers during intrusive growth, showing only a nucleus, LS.

(Co, corpus; F, fiber; Pc, procambium; Tu, tunica; V, vessel). – Scale bar for a, c, d- i = 10µm, for b =100µm
longer than the neighboring ones and appeared deeply stained with safranin.

Transversely, the vascular bundles were to differentiate from procambium cells with the culm elongation. The procambium strands had only four cells formed early (Fig. 2, c), and then divided into a cluster (Fig. 2, d). With further development, protophloem sieve tubes from the outer layers of procambium appeared (Fig. 2, e). Afterwards, the first protoxylem vessel, the annular vessel, developed from the inner layers of procambium (Fig. 2, f). With internodal elongation of the culm, metaxylem vessels from the lateral cells of procambium and vascular bundle parenchyma from the middle ones gradually differentiated. Nevertheless, a layer of procambium cells to form the culm fibers was still around the vascular bundle elements as fiber initials (Fig. 2, g).

**Primary wall formation**

During primary wall formation, a fiber successively exhibits co-growth with vascular elements and intrusive growth.

At this stage, fiber maintained its cylinder form and partly elongated coupled with radial extension and elongation of vessel elements. Bi-nuclei or multinuclei cells were observed with a dense protoplast and smaller vacuole (Fig. 2, h). Following, fibers with bi-nuclei or multinuclei were gradually vacuolated and in parallel way arranged.

When the co-growth of fiber terminated, fibers longitudinally arranged in stagger way were fusiform, indicating the beginning of intrusive growth. At this stage, a central large vacuole and only a single nucleus with distinct nucleolus were seen (Fig. 2, i). When fiber walls were stained red by safranin due to lignification, primary wall formation came to an end.

During primary wall formation, fiber underwent centrifugally differentiation and development transversely. Earlier only a layer initials surrounded the vascular bundle. Later they radially divided into two daughter cells. Thereafter, the outer ones maintained its meristematic character, while the inner ones increasingly vacuolated and elongated until the end of fiber primary wall formation (Fig. 3, a). Similarly, fiber cap formed increasingly (Fig. 3, b).

**Secondary wall formation**

During secondary wall formation, fiber walls lignified and thickened with aging. During the first 4 years, a dominant thickening of fiber wall was observed (Fig. 3, c-e). Later, the degree of thickening decreased gradually.
Fiber wall is characterized by a regular alternation of broad and narrow lamellae during thickening, as also observed by Parameswaran & Liese (1976, 1980).

With the formation of secondary wall, fiber nuclei underwent a series of changes. Almost all fiber nuclei with distinct nucleoli in one-year-old culms were fusiform and unevenly stained (Fig.3, g). In contrast, the nuclei in the fibers adjacent to the vascular bundle were evenly stained with crystal violet, due to the agglutination of chromatin (Fig.3, f). With the continuous thickening of fiber wall, the nuclei became strip-formed and evenly stained. They were persistent for eight years (Fig.3, h).

Ultrastructural investigations showed the plasmodesmata persistent in the border pit pairs between fibers and their adjacent cells during secondary wall formation. A great number of transfer vesicles were also seen in the pit channels (Fig.3, i).

**DISCUSSION**

**The origin of fibers**

The fibers in the culm of *Phyllostachys edulis* around a vascular bundle form a fiber cap or fiber sheath, but their origin is still controversial. Fahn (1982) considered that the inner cells of the fiber cap originated from procambium, but the outer ones from ground meristem. According to Xiong et al. (1980b), procambium cells first differentiated into parenchyma just around the vascular elements, and then differentiated into fiber cells, so that fibers originate from parenchyma around vascular bundle. In this research, fiber cells differentiated later than vascular tissue. With the formation of vascular bundle elements, the cells just around can become fiber initials. Subsequently, they underwent differentiation and development centrifugally outwards from vascular bundle resulting in the formation of fiber caps. Whereby the development degree of fiber in a fiber cap decreased outwards, and the wall thickness of inner cell was thinner than at the outer part. Accordingly, fiber cells originate from the same procambium strand as vascular bundle elements.

**The bi-nucleate or multi-nucleate phenomenon of fibers during primary wall formation**

During primary wall formation, fiber cells were bi-nucleate or multi-nucleate, while the phenomenon disappeared with secondary wall formation. Bi-nuclei or multi-nuclei were first reported by Esau (1943) in the development of the primary phloem fibers of *Nicotiana* and *Linum*, and also by Xi and Bao (1997) in the fusiform initial cell and ray initial cell of *Camptotheca acuminata* cambium. Hu and Zhu (2000) thought that bi-nucleate or multi-nucleate occurrence is related to amitosis, which enables faster gene amplification in a cell. The bi-nuclei or multi-nuclei can provide more nutrients for the development of the pollen strengthening the metabolism. Xiong et al. (1980a) discovered the phenomenon of amitosis in internodes elongation, but did not discuss the relationship between amitosis and the bi-nucleate or multi-nucleate phenomenon. During the growth of *Phyllostachys edulis* culm, internodal elongation attributed to amitosis leading to the occurrence of bi-nuclei or multinuclei. Fiber primary wall formation and elongation is consistent with the internodal elongation of culms. We presume that the bi-nuclei or multi-nuclei of a fiber can strengthen its metabolism to meet the demands of fiber elongation. When fiber elongation came to an end, amitosis was unnecessary as shown in the disappearance of bi-nuclei or multinuclei. How the bi-nuclei or multi-nuclei phenomenon of fiber disappeared was not investigated in this paper. The mechanism of nucleus change in bamboo fiber has to be studied further during fiber development.

**The variation of fiber wall**

The variation of fiber wall was largely reported to date. Fujii (1985), reported that larger fibers of *Pleioblastus chinensis* continued thickening late into the second year. Alvin and Murphy (1988) found that fiber walls in *Sinobambusa tootsik* were to go on thickening into the third year. Murphy and Alvin (1992) discovered that fiber walls in the 3-5 years old culms of *Phyllostachys viridi-glaucescens* had a polylamellate structure. Liese and Weiner (1996), in a valuable view of ageing in bamboo
culms, showed that the culm of *Phyllostachys viridi-glaucens* underwent aging processes involving fiber wall thickening. While in their research (Liese & Weiner 1997), fiber wall thickness of the same bamboo was observed to increase during the maturation period, but also up to the investigated 12 years. Most recently, Lybeer and Koch (2005) investigated the lignification during ageing of bamboo culm. However, the variation law of fiber wall with aging was unclear as yet.

The result in our investigation just indicated the variation law of fiber walls during development: the fiber wall underwent a fast thickening in the first 4 years, and then the degree of thickening decreased. The variation of fiber wall is consistent with the growth of bamboo culms (Zhou, 1998): in the first 4 years, the growth of bamboo culms was dominant, and then the degree of culm growth gradually decreased with aging. That means the fibers of *Phyllostachys edulis* culms underwent co-development with the culm growth. Only the variation of fiber wall was qualitatively analyzed in our research. Further studies about quantitative investigation of wall variation and physiological analysis during fiber development will help us to reveal the variation mechanism of fiber wall.

**The persistence of the fiber nucleus and plasmodesmata**

In this study, almost all fibers in the middle third of a one-year-old culm wall of *Phyllostachys edulis* still had nuclei with a distinct nucleolus, and then their nucleoli gradually disappeared, while the nuclei of fibers persisted for up to eight years. In addition, the persistence of plasmodesmata and transfer vesicles could guarantee intercellular linkage between the fibers and their adjacent cells. The results showed that the fibers in the middle third of *Phyllostachys edulis* culm walls kept their living protoplast for a long time after lignification and wall thickening. Generally, a mature fiber is considered a dead cell without protoplast, whereby bamboo fibers can maintain their protoplast for many years (Liese, 1998). In addition, it was reported that the xylem fibers of *Tamarix aphylla* kept alive for 20 years (Fahn, 1982).

Due to secondary growth of woody dicotyledons, new secondary xylem will form and become sapwood instead of old secondary xylem. Fibers with living protoplast are usually in sapwood, and have functions in sustaining and storing (Gu et al., 1993), while those in heartwood, losing their protoplast, become dead cells. Different from other woody plants, bamboo in its absence of secondary growth depends mainly on its primary vascular system during the whole life of a culm. Once differentiating from procambium, bamboo fibers as an important component of primary vascular system will remain alive for many years. The persistence of nuclei and intercellular linkage attributes to continuous thickening of fiber secondary wall. This obviously shows that the fiber studied in this research is a special long-living cell, quite different from fibers in dicotyledons.

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