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RESEARCH ON THE STRUCTURE AND CONNECTIONS OF PITS IN DIFFERENT CELLS OF MOSO BAMBOO (*PHYLLOSTACHYS PUBESCENS*)

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ABSTRACT

The plant grows within the transportation of water and nutrients, including radial and longitudinal, but bamboo only exists pits in the radial, so it plays an irreplaceable role at this moment. This study aims at giving rise to further understanding of the biological functions of pits in bamboo. Light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were applied to investigate the structure and connections of bamboo pits. The results show that the arrangement of pits is significantly different, including alternate, scalariform and opposite arrangements. The presence or absence of the bordered on different cells is also displayed distinctively, these characteristics extremely affect the transportation of water and nutrients in bamboo.

KEYWORDS: Bamboo, pit, vessel, parenchyma cells, fibers.

INTRODUCTION

Bamboo is considered to be one of the fastest-growing plants in the world, it relies on a special rhizome-dependent mechanism (Hamzah et al. 2016, Ahmad et al. 2021). The culm can grow to its full height of 3-30 m within only 2-3 months. It has been confirmed that the rapid growth of the culm results from the fast growth of its individual internodes (Liese 1998, Akinlabi et al. 2017, Wei et al. 2019). In the beginning, the whole internode consists of an intercalary meristem which is formed after the primary meristem is separated by nodes. Heterogeneity starts with the elongation of different cell types. The whole process of the heterogeneity of one internode is completed in only a few days (Liu et al. 2021), this growth characteristic is inseparable from the transport of water and nutrients.

Increased research during recent years has contributed greatly to our understanding of the mechanism of the rapid growth, such as the water and nutrient transport system of bamboo

(Zwieniecki et al. 2015, Zhan et al. 2020, Yang et al. 2021, Fuke et al. 2021). At the internode, the cells are axially oriented. The water and nutrients can transport freely through the vessels which consist of short cell elements connected by perforates (Kaur et al. 2019). However, in contrast to trees, the internodes do not have any radial cell elements, such as radial rays (Shao et al. 2018, Wang et al. 2020, Xu et al. 2021). The movement of water and nutrients in the transverse direction in the internode is mainly across the pits on the cell wall (Su et al. 2021).

As the unthickened portion of the primary wall, pits play an indispensable role in the relationship between plant growth and the external environment. Meanwhile, the pores in the pit membrane can limit the spread of embolism and vascular pathogens in the xylem (Jansen et al. 2001, Hong et al. 2017, Zhang et al. 2019). The structure of pits varies dramatically with large differences in the arrangement and pit pairs types (Choat et al. 2008). Half-bordered pits were found between parenchyma cells and the adjoining vessels of bamboo, mostly in alternate or opposite arrangement, the fibers were connected with each other by bordered pits. Moreover, pits also affect the penetration of liquids and preservatives in bamboo (22et al. 2019). However, the structure and connections of pits in the different cells of bamboo are still unclear.

In past studies, researchers worked on individual tissues, such as metaxylem and parenchyma cell, SEM is the main instrument, resin castings is the normal approach, the qualitative and quantitative characteristics of pits on bamboo were found (Luo et al. 2019, 2020, Lian et al. 2019, 2020). However, there are rare studies that compare the differences between pits on various cells, and the way of the water transporting in adjacent cells is unknown. Due to the lack of detailed information about bamboo pits, the aim of this paper is to study the structure and connections of pits in the different cell walls of bamboo by light microscopy, scanning electron microscopy and transmission electron microscopy, to give rise to further understand the biological functions of pits in bamboo.

MATERIAL AND METHODS

Materials

Moso bamboo (*Phyllostachs pubescens*) at one year old was obtained from Huangshan, Anhui Province, China. The specimens were cut from the internode located at the height of about 1.5 m and were mostly air-dry at room temperature.

Methods

For light microscopy, it is based on the approach adopted by Lian et al. (2020), bamboo specimens with the size of 20× 20× 5 mm were cut from the internode. The specimens were put in the solution of acetic acid and 30% hydrogen peroxide (1: 2, v/v) for 3 days. Then they were cooked with hot water and sectioned to a thickness of 20-30 μm using a sled microtome (Reichert, Austria). After being bleached, the sections were stained with the solutions of 1% safranin in 50% alcohol and 1% aqueous alcian blue for 20 sec before being taken through an alcohol series (50%, 70%, 96%, 100%). The sections were put to dimethyl benzene for a few seconds, then transferred them to the slides and embedded them by Canada balsam. The slides were placed in the oven at 60°C for 2 weeks. The morphological features were observed using a Zeiss V20 light microscope (Zeiss, Germany) and pictures were taken using an AxioCam digital camera (Zeiss, Germany).

For scanning electron microscopy, it is based on the approach adopted by Schulte et al. (2015) and Lian et al. (2020), bamboo specimens with the size of 20 mm × 20 mm × 1.5 mm were cut from the internode. The surfaces of the specimens were trimmed by a sled microtome (Reichert, Austria). Next, the specimens were bleached by the solution of NaClO for 1 min, and they were washed with distilled water three times. Then, the specimens were dehydrated in an ethanol series of 50%, 70%, 96%, 100% for 5-30 min at each concentration level. Finally, the specimens were put onto the stage and sputter-coated with 10 nm of gold (SPI Supplies, USA). Samples were observed using a scanning electron microscope operated at 7 kV (JSM 7600F, JEOL, Japan).

For transmission electron microscopy, it is based on the approach adopted by Jansen et al. (2012) and Zhang et al. (2017), bamboo specimens with the size of 2.5 mm × 2.5 mm × 5 mm were cut from the internode under a microscope. The specimens were put in the Karnovsky buff for one week. To enhance the contrast during TEM observation, the specimens were treated with 1% osmium tetroxide for one night, then the specimens were dehydrated with a graded ethanol series (50%, 70%, 96%, 100%) with uranyl acetate solution for 1 hour at each step. The specimens were placed to the solution series with the propylene oxide and phenolic epoxy resin (1:0, 2:1, 1:1, 1:2, 0:1, v/v) for 2 hours at each step. The specimens were embedded in the confirmation by new epoxy resin and placed in the confirmation holder in the oven at 60°C for 48 hours. After being trimmed, ultra-thin transverse sections were cut from embedded material with a diamond knife Diatome (ultra45°) on a Leica ultramicrotome (UC7, Leica, Germany) and stained with uranyl acetate and lead citrate. Photographs were taken under a transmission electron microscope (JEM-1010, JEOL, Japan) at an acceleration voltage from 60 to 100 kV.

RESULTS AND DISCUSSION

Microscopic structure of bamboo

Bamboo, as a typical vascular plant, consists of ground tissue and vascular bundles (Parameswaran et al. 1976, Dixon et al. 2014). As shown in Fig. 1, the parenchymatous ground tissue consists of parenchyma cells, with embedded vascular bundles composed of metaxylem vessels, sieve tubes with companion cells, and fibers. It is obvious that the vessels are surrounded by parenchyma cells, even in the area connecting to the fiber sheaths. The morphological characteristics of the cross-sections of the bamboos are relatively simple, almost all of them are round. Moreover, the phloem consists of large sieve tubes and smaller companion cells.

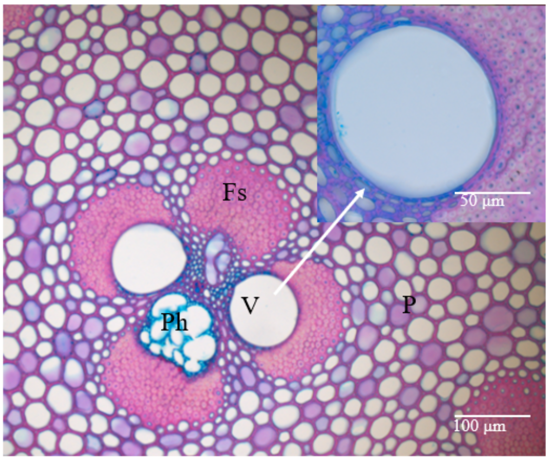

Fig. 1: Light microscopy cross-section photograph of bamboo structure (V - metaxylem vessels, Ph - phloem, Fs - fiber sheaths, P - parenchyma.

Pits distribution of different cells

Pits are narrow cavities through the thick secondary cell walls, which provide the main pathway for the interconnection of fluids and other substances between cells. There are three types of the arrangement of pits, namely alternate, scalariform and opposite. The primary function of the vessels is to provide a pathway for water movement through the plant. Each vessel consists of numerous vessel elements which are connected end-to-end by perforation plates to form mainly pathways for water movement in a longitudinal direction. However, the structure of bamboo is considerably different from that of wood, there are no radial cell elements, and water moves between vessels and adjacent parenchyma cells must through the pits on the lateral wall (Donaldson et al. 2018). As shown in Fig. 2, abundant pits were found on both radial and tangential walls of the vessels. They were mostly in alternate or scalariform arrangements and occasionally in the opposite. The shape of the pits in the inner lateral wall was most often either flat and elliptical or with long, narrow slits.

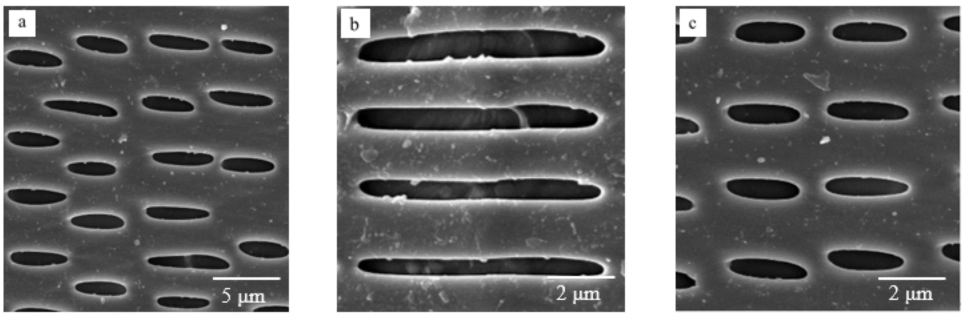

Fig. 2: SEM photographs of pits morphology on the inner wall of vessel: a) alternate pits, b) scalariform pits, c) opposite pits.

In plants, the parenchyma cells mainly perform storage functions. These cells can store carbohydrates in the form of starch (Trifilò et al. 2019). The parenchyma cells of bamboo have shown diversification, consisting of the ground parenchyma cells and the parenchyma cells surrounding the vessel. The latter are generally smaller than the former (Palombini et al. 2020). The pits of these parenchyma cells are mostly in alternate arrangements and occasionally in the opposite (Figs. 3a,b). The ground parenchyma consists of two types of parenchyma cells, the vertically elongated cells and short cube-like ones interspersed among the former (Fig. 3c), this is the same as the discovery of He et al. (2002) and Lian et al. (2019). The pits of these parenchyma cells are mostly in separate or alternate arrangements. Therefore, we speculated that the function of parenchymal cells in vascular bundles is mainly to connect the two transportation tissues of vessels and sieve tubes so that water and nutrients can flow freely and interactively in the basic tissue parenchymal cells, vessels, and sieve tubes.

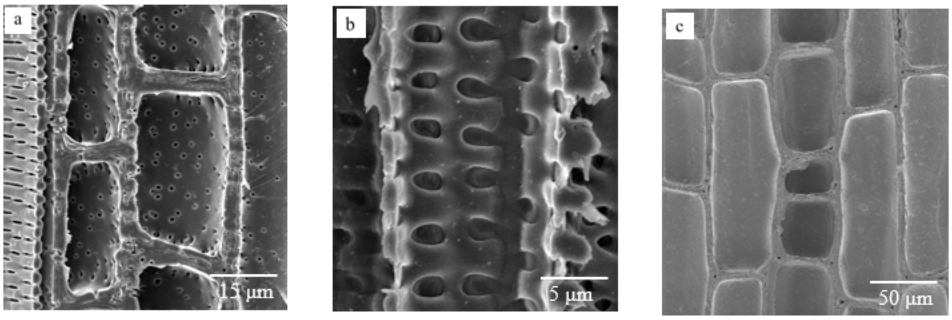

Fig. 3: SEM photographs of pits morphology on the wall of parenchyma: a) the pits morphology of the parenchyma cells surrounding the vessel, b) opposite pits of the parenchyma cells surrounding the vessel, c) two types of parenchyma cells in the ground tissue.

There were only a few to very few small sample pits on the fiber. Sometimes only one pit is observed on the fiber (Fig. 4), these results were consistent with the conclusion of a previous study (Parameswaran et al. 1976, Gan et al. 2004). There may be something to do with the growth and development of bamboo, in the development process of bamboo fiber, due to the continuous thickening of the secondary wall, the pits channels gradually become longer and narrower, and the pits cavities gradually become smaller, and finally develop into simple pits. Moreover, the microfibrils around the pits run in a streamlined fashion. In contrast to vascular and parenchyma cells, fibers provide mechanical support for the culm as fiber sheath or fiber bundle. The fewer and smaller pits can improve the excellent mechanical properties of bamboo (Lian et al. 2020).

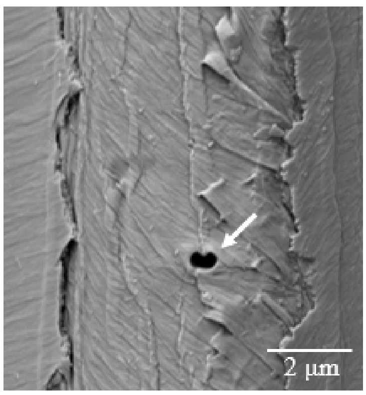

Fig. 4: SEM photographs of pits morphology on the wall of fiber.

Pits connection between different cells

According to the presence or absence of the pit margin, pits are divided into two types: namely simple pits and bordered pits, meanwhile, pits often exist in pit pairs, there are simple pits pair, half-bordered pits pair and bordered pits pair. As shown in Figs. 5a,b half-bordered pit pairs are found between the vessel and the surrounding parenchyma cell, however, the pits pairs of the fiber and the parenchyma cells surrounding the vessel are typically bordered pits, which have a secondary wall arching over the pit cavity. Figs. 5c,d,e show the pits connections between the parenchyma cells which are located in the metaxylem, protoxylem and the ground tissue, they are mostly simple. The research on the connections of pits between various cells has made up for the previous blank, it is not only an important way for two adjacent cells to communicate water and nutrients but also closely related to its processing and utilization, such as bamboo drying, flame retardant modification and immersion treatment.

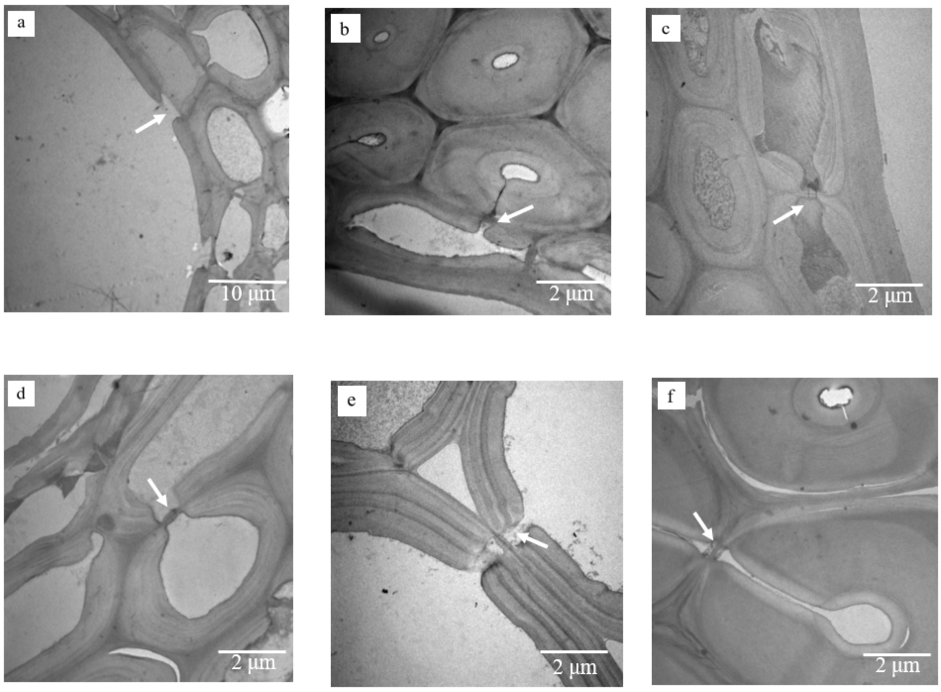

Fig. 5: Pits connections of bamboo by TEM: a) half-bordered pit pair between the vessel and the parenchyma cell surrounding the vessel, b) bordered pit pair between the fiber and the parenchyma cell surrounding the vessel, c) simple pit of the parenchyma cells which are located in the metaxylem, d) simple pit pair between the parenchyma cells which are located in the protoxylem, e) simple pit pair between the parenchyma cells which are located in the surrounding of the ground tissue, f) bordered pit pair between the fibers.

The pit cavity remains of the same diameter, no such arching structure of the bordered occurs. Moreover, the pits of ground parenchyma cells are smaller than the parenchyma cells which surround the vessels Fig. 5f shows the pits connections of fiber. The pits between fibers are typically bordered. However, the pit cavity and border are much smaller than the vessels and parenchyma. These results indicated that the possibility of bordered depends on the function of diverse cells, the characteristics, including the difference of the cell morphology and the diversity of the pit structures, influenced the physiological function of bamboo storage and transportation.

CONCLUSIONS

In summary, the structure of pits makes the bamboo cells transversely connected, forming a coordinated organism. The pits of the vessel are typically bordered, mostly in alternate or scalariform arrangement and occasionally in the opposite. Half-bordered pit pairs are found between the vessel and the surrounding parenchyma cells. The pits of the parenchyma cells are mostly in alternate or separate, and occasionally in opposite arrangements. Bordered pits pairs are found between the parenchyma cells which surround the vessel and fiber. While simple pit pairs are present between the parenchyma cells which are located at the intercellular space of the protoxylem and ground tissue. Few to very few small bordered pits are found on the wall of fibers. Together, these findings increased our understanding of the pits, it is very important to study the transportation of water in bamboo and can serve as the theoretical and data basis for future improvements of bamboo production and utilization.

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