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NOTE

Rhizome and root anatomy of moso bamboo (*Phyllostachys pubescens*) observed with scanning electron microscopy

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Abstract In this study, we observed the rhizome and root anatomy of moso bamboo (*Phyllostachys pubescens*) with scanning electron microscopy compared with the anatomy of the culm. The epidermis of culm, rhizome, and root were hard multi-layered and composed silica cells. The culm and rhizome consist of the epidermal, parenchyma, and vascular tissues. Although features of the anatomical structure of the rhizome were similar to those of the culm, the shape and distribution of vascular bundles and parenchyma differed between the organs.

Keywords Moso bamboo · Rhizome · Root · Scanning electron microscopy · Anatomy

Introduction

Bamboo forests in Asia have long been managed to provide bamboo culms, which are used as materials for ornaments and building, and bamboo shoots, which are popular in Asian cuisine [1]. In recent years, research has focused on the development of bamboo for additional purposes, such as charcoal, vinegar, and fibers, in addition to conventional applications [2–4]. However, the target of these studies is the use of bamboo culms. Less detailed information is available on the anatomical structure and chemical composition of bamboo rhizomes and roots, although structural features of bamboo culms [5, 6] and seasonal changes in the contents of biochemical components [7–9] have been studied.

In addition, the bamboo forests that are no longer actively managed are spreading rapidly in many areas of Japan. The strong growth of bamboo allows it to invade surrounding forests [10]. The spread of bamboo also threatens agricultural lands, and can lead to poor biodiversity and the risk of landslides [11, 12]. Removing the rhizomes with the culms is an effective method to prevent the spread of bamboo forests, since bamboo propagates from the rhizomes. Thus, it is important to determine their fundamental characteristics to use them effectively. In our previous study on the seasonal change of chemical components in rhizomes, the contents of mono- and polysaccharides such as starch, sucrose, fructose, and glucose increased from winter to spring [13]. In the present study, to explore the structural features of rhizomes and roots, we examined the anatomical structure of the rhizome and root of the most common bamboo species in Japan, moso bamboo (*Phyllostachys pubescens*), using scanning electron microscopy (SEM).

Materials and methods

Plant materials

Rhizomes and roots of moso bamboo (*P. pubescens*) were collected on 29 August 2012 from the bamboo forest in Takagamine, Kyoto. This forest is a part of Kyoto Prefectural University, located at 35°3′17″N and 135°43′30″E. The dominant species in this forest is moso bamboo, with an approximate density of 4000 culms/ha. The soil was carefully removed from the rhizomes by washing with water. Nodal roots were selected for examination. The

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cleaned rhizomes, roots and culms were stored at −18 °C until observation. Four-year-old culms were collected on 20 November 2012 from the same forest. Growth of current year culm is not enough yet and the wall of bundle sheath fiber in it is thin [14]. Therefore, 4-year-old culms were selected for examination to compare of rhizome and culm.

Scanning electron microscopy

The rhizomes, roots, and culms were cut into block samples [approximately 5 mm (radial) × 5 mm (tangential) × 5 mm (longitudinal)]. The samples were surfaced with a sliding microtome. After drying for 24 h at 105 °C, each specimen was mounted on a specimen holder and then coated with gold. The exposed surface was examined with a SEM (JFC-1600, JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. Size of observed five tissues and cells which were chosen randomly from SEM images were measured by image analysis software (Motic Image Plus 2.2S, Shimadzu, Kyoto, Japan), and their averages and standard deviations (SD) were calculated.

Scanning electron microscope-energy dispersive X-ray (SEM-EDX) analysis

We conducted elemental analyses to analyze the composition of grains in bundle sheath cells. The sample was examined under a field emission-scanning electron microscope (FE-SEM) (S-4800, Hitachi) coupled with an energy dispersive X-ray analyzer (EDX) (Genesis XM2, EDAX, Japan).

Results and discussion

Comparison of rhizome and culm anatomy

The SEM images of the vascular bundles of the rhizome and culm of moso bamboo are compared in Fig. 1. In both organs, the vascular bundles were composed of metaxylem, metaphloem, and bundle sheaths, although the size and number of bundle sheaths differed slightly (Fig. 1a–e). Vascular bundles near pith hole of the culm have four bundle sheaths. Two of them are on either side of the vessels, one is around the phloem and the other one is around the intercellular space. This style is consistent with the results in the previous reports [15]. However, vascular bundles near pith hole of the rhizome have two bundle sheaths. One is around the vessels and another is around the phloem. Tylosoids were observed in the culm (indicated by arrows in Fig. 1d, e) but were barely discernible in the rhizome (indicated by arrows in Fig. 1a, b). In the previous report, tylosoid was found in moso bamboo culm [16]. Tylosoid is defined as follows: an outgrowth of parenchyma cells or epithelial cells. Evert reported that tylosoids exit as outgrowths of parenchyma cells into sieve elements in phloem and of epithelial cell into intercellular resin ducts [17]. The lumen of bundle sheath fibers in the

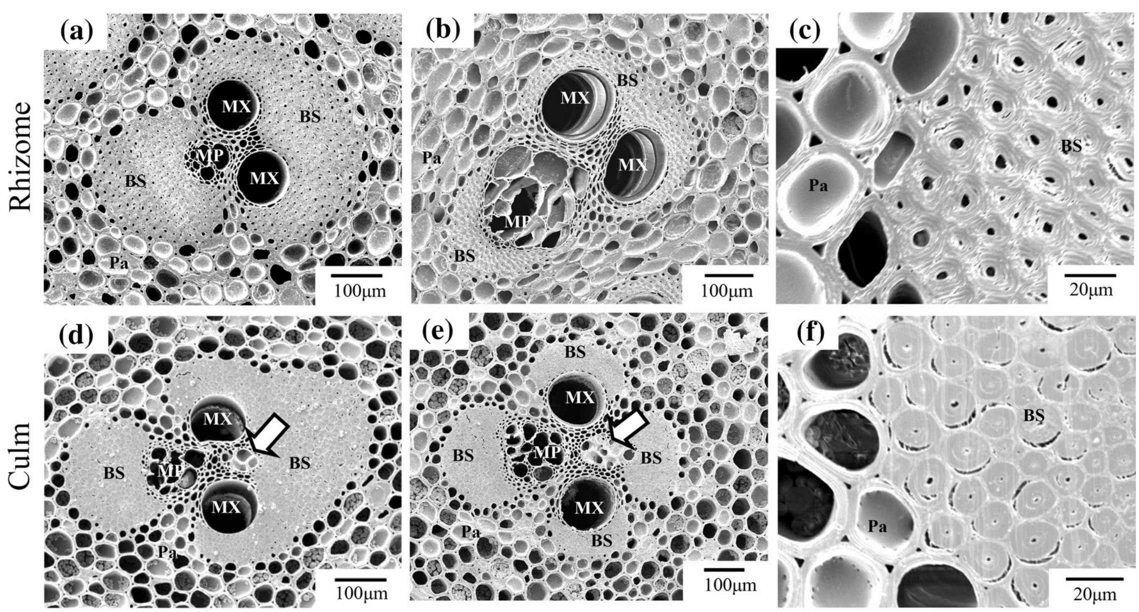


Fig. 1 SEM images of vascular bundles in the rhizome (*upper*) and culm (*lower*). **a**, **d** Vascular bundle near the epidermis; **b**, **e** vascular bundle near the pith cavity; **c**, **f** cortical parenchyma and bundle sheath fibers. *MX* metaxylem, *MP* metaphloem, *BS* bundle sheath, *Pa* parenchyma, *arrow* in **d** and **e** tylosoid

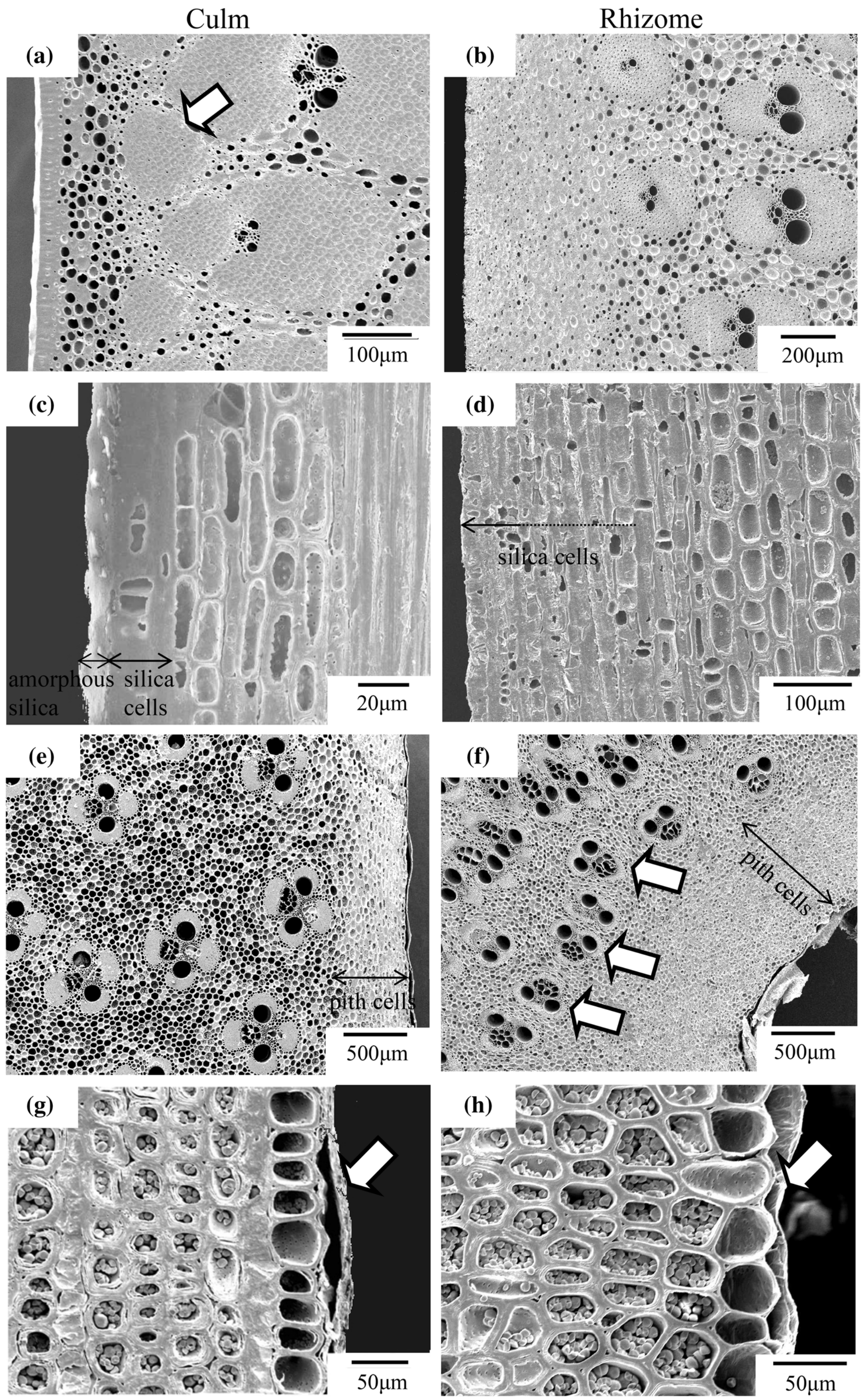
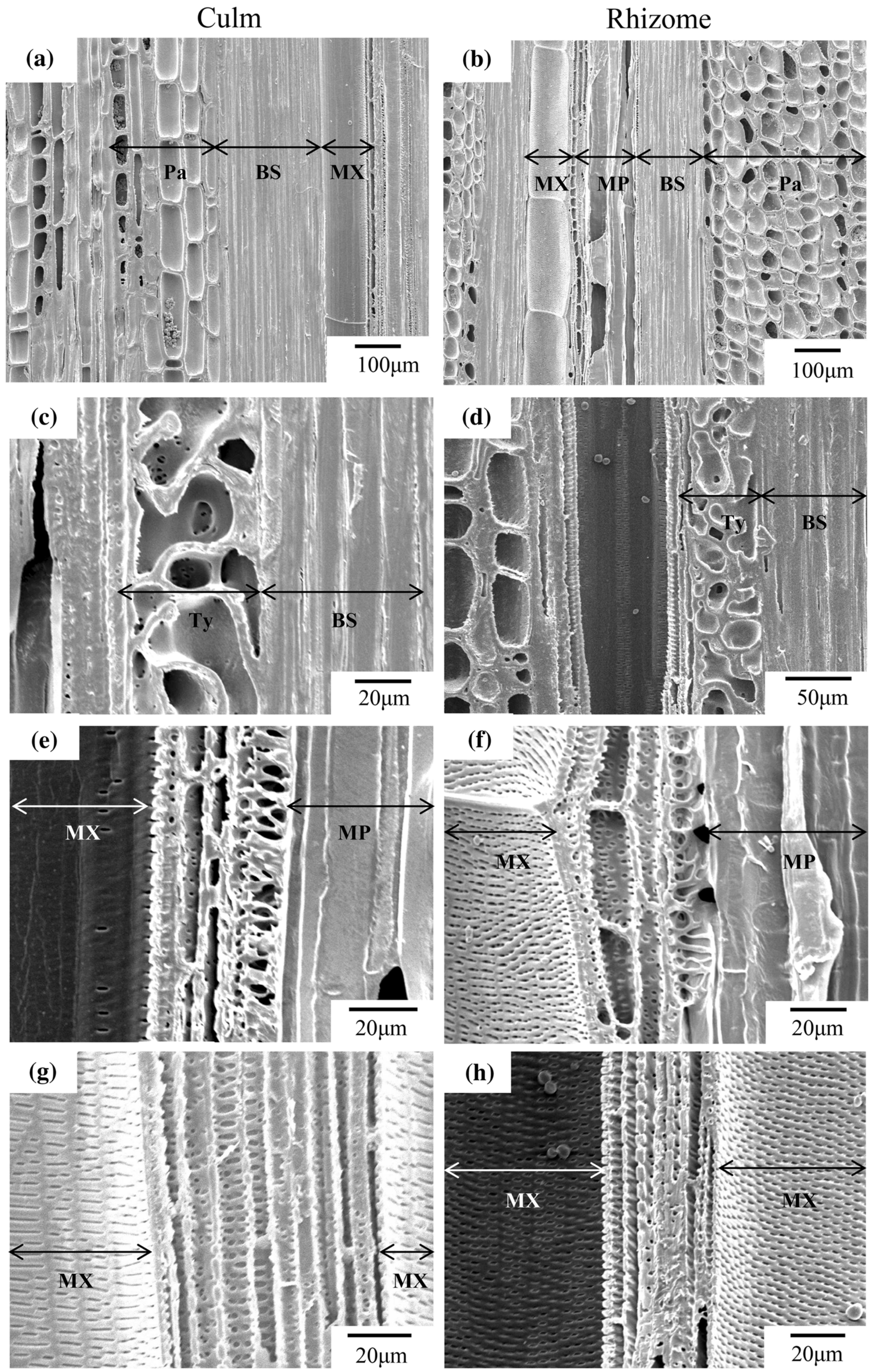


Fig. 2 SEM images of the epidermis and margin of the pith cavity in the culm (*left*) and rhizome (*right*). **a**, **b** Epidermis and outer cortex in transverse section; **c**, **d** epidermis in radial section; **e**, **f** margin of pith cavity in transverse section; **g**, **h** margin of pith cavity in radial section. *Arrow* in **a** fiber bundle, *arrow* in **f** orientated vascular bundle irregularly, *arrow* in **g** and **h** pith paper

culm was smaller than that of bundle sheath fibers in the rhizome (Fig. 1c, f). Diameter of the lumen in rhizome was 4.0 ± 1.7 µm, although that of culm was 0.8 ± 0.5 µm.

Fiber bundles were present among the cortical parenchyma between the epidermis and vascular bundles in the culm (indicated by arrow in Fig. 2a). The distance

Fig. 3 SEM images of longitudinal sections of the culm (*left*) and rhizome (*right*). **a**, **b** Parenchyma and vascular cells in radial section; **c**, **d** tylosoid in tangential section; **e**, **f** metaxylem and metaphloem in radial section; **g**, **h** inter-vessel cells in tangential section. *MX* metaxylem, *MP* metaphloem, *BS* bundle sheath, *Pa* parenchyma, *Ty* tylosoid



between the epidermis and vascular bundles in the rhizome was larger than that of the culm (Fig. 2a, b). The distance of rhizome was 457.6 ± 28.9 μm, although that of culm was 112.9 ± 2.9 μm. Typically, the culm epidermis in bamboo contains rigid silica cells in which the lumen is almost entirely filled with silicon dioxide [18]. These were covered with amorphous silica. Although the epidermis of both the rhizome and culm comprised silica cells, in the

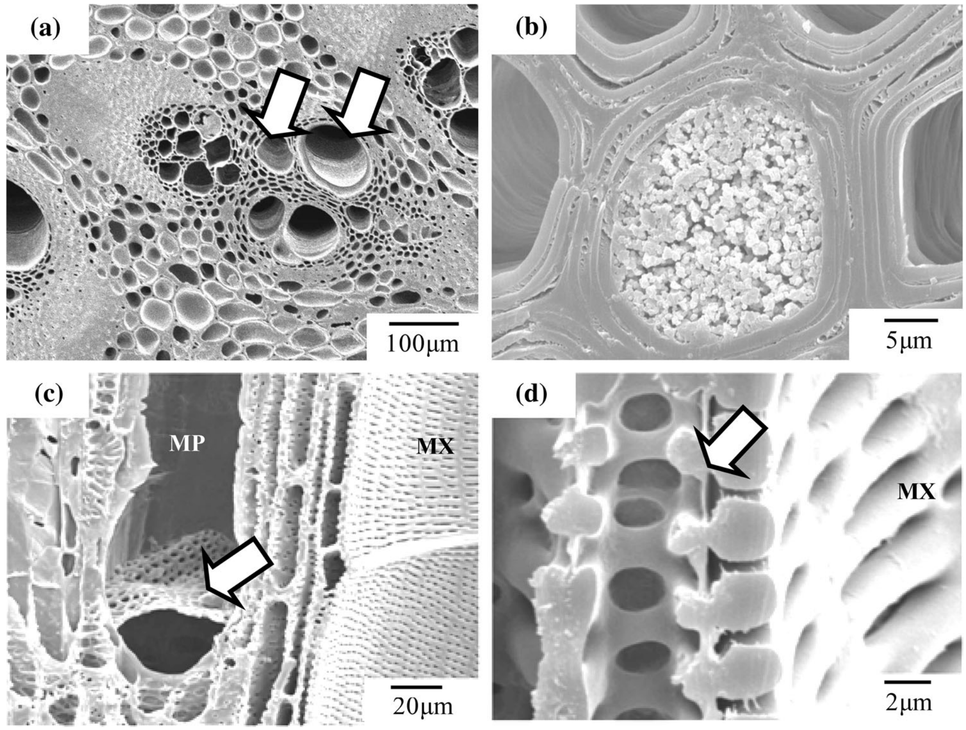


Fig. 4 SEM images of vascular tissues in the rhizome. **a** Unusual vascular bundle structure in transverse section, **b** grains in bundle sheath fiber in tangential section, **c** sieve plate in metaphloem in tangential section, **d** reticulate vessel pits in tangential section. *MX* metaxylem, *MP* metaphloem, *arrow* in **a** two pairs of metaxylem vessels, *arrow* in **c** sieve plates, *arrow* in **d** intervascular pit membrane

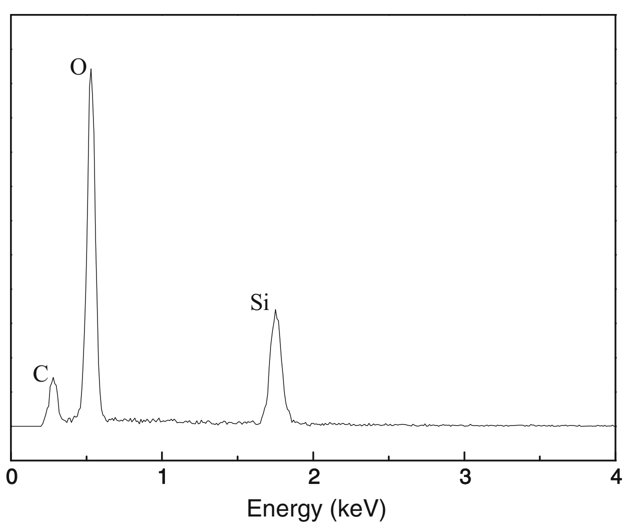


Fig. 5 EDX spectrum of grains in bundle sheath

rhizome the band of silica cells was broader than that of the culm (Fig. 2c, d). The band of rhizome was 200 μm or more, although that of culm was 28.3 ± 3.4 μm. Silica prevents various fungal and bacterial diseases, and also alleviates the effects of various abiotic stresses such as salt stress, metal toxicity, drought stress, high temperature, and freezing [19]. It is possible that silica in the rhizomes of bamboo has similar effects. The vascular bundles nearest the central piths cavity in the culm arranged with regularity such that the metaxylem is at the pith side and the metaphloem is at the opposite side (Fig. 2e), whereas those in the rhizome showed irregular orientation (Fig. 2f). The layer of pith cells in the rhizome was thicker than that in the culm (Fig. 2e, f). The thickness of the layer in rhizome was 925.2 ± 46.8 μm, although that of culm was 473 ± 41.3 μm. The pith cells in both the rhizome and culm contained many starch grains, although the innermost cell layer in both the rhizome and culm contained few starch grains (Fig. 2g, h). In addition, both the rhizome and culm contained a sheet of pith hole paper (indicated by arrows in Fig. 2g, h).

Parenchyma cells in both the rhizome and culm were axially aligned. Although the parenchyma cells in the culm were barrel shaped, those in the rhizome where spherical (Fig. 3a, b). Tylosoids in both the rhizome and culm were foamy, but the tylosoid cell wall in the rhizome was thicker than that in the culm (indicated by arrows in Fig. 3c, d). The thickness of the cell wall in rhizome was 5.5 ± 0.6 μm, although that of culm was 4.8 ± 1.2 μm. The metaphloem in the rhizome and culm contained no pits in the cell walls. However, in the cells situated between metaxylem and metaphloem elements, many pits in the cell wall were observed (Fig. 3e, f). The cells located between vessels were tubular with many pits in the cell wall (Fig. 3g, h). These cells with many pits might assist with the water-transporting function of the metaxylem.

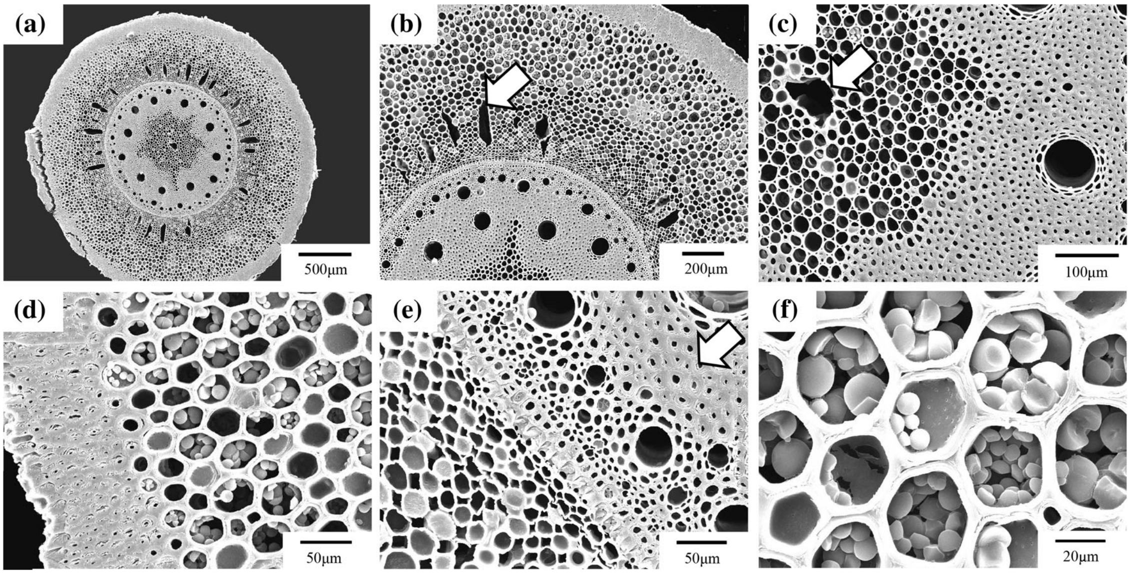


Fig. 6 SEM images of transverse sections of the root. **a** General view, **b** cortex, **c** pith, **d** epidermis, **e** endodermis, **f** cortical parenchyma, *arrow* in **b** aerenchyma, *arrow* in **c** pith cavity, *arrow* in **e** cell which has thickened cell wall

The features in the vascular bundles, bundle sheaths, metaxylem, and metaphloem were observed in the rhizome (Fig. 4a–d). Some vascular bundles in the rhizome contained two pairs of metaxylem vessels (indicated by arrows in Fig. 4a). Grains unlike starch grains are in the several bundle sheath cells (Fig. 4b). From EDX spectrum of the grains in bundle sheaths (Fig. 5), these are composed Si and O mainly indicating grains shown in Fig. 4b are silica. In addition, the bundle sheath fiber wall comprised multiple layers (Fig. 4b). These tissues in Fig. 4a and b are unusual. In the previous report, sieve plates were found in moso bamboo culm [16]. Sieve plates were also observed in the metaphloem (Fig. 4c). The metaxylem vessel walls were reticulate with simple pits which were partitioned by intervascular pit membranes (indicated by arrow in Fig. 4d).

Observation of root

In the transverse section, the root was similar in shape to that of typical monocotyledons (Fig. 6a). Aerenchyma was observed in the cortex (Fig. 6b). A pith cavity was present in the center of the root as in the culm and rhizome (Fig. 6c). The root epidermis was composed of silica cells, which contained a thick cell wall similar to that of epidermal cells in the culm and rhizome (Fig. 6d). The cells located between the phloem and pith had a thickened cell wall similar to that of bundle sheath fibers in the rhizome and culm (indicated by arrow in Fig. 6e). The cortical parenchyma cells contained many starch grains (Fig. 6f).

Conclusions

The culm, rhizome, and root of moso bamboo are covered with a hard multi-layered epidermis containing abundant silica cells. The culm and rhizome consist of epidermal, parenchyma, and vascular tissues, whereas the root also contains aerenchyma and a band of thickened cells between the phloem and pith. The rhizome differs anatomically from the culm in shape and in the orientation of the vascular bundles, the wall thickness of bundle sheath fibers, and layer structure near the pith hole. Consequently, the rhizome and culm are notably different in morphology from that of the root.

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