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Research article

Ultrastructure of Bamboo (*Bambusa vulgaris* Schrad. ex J.C. Wendl.)

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Abstract: Micro-structures of bamboo (*Bambusa vulgaris*), a source of innumerable uses to man for ages were investigated in this study. *B. vulgaris* lacks secondary thickening as opposed to what is obtained in trees, but possesses numerous vascular bundles consisting of xylem, phloem and fibre caps which are embedded in parenchymatous tissues. Both light microscope and scanning electron microscope were used in this study. Transverse sections of about 20 micrometers thick were prepared on a Reichert microtome. Slides were examined under a Zeiss microscope (Standard 25) ×80 magnification, attached to a digital camera. Electron microscopy was performed using small blocks (3 mm^2) attached to stubs using electron-conductive carbon paste. The samples were sputter-coated for 3 min with gold. The results showed that each vascular bundle was composed of a protoxylem vessel, two metaxylem vessels and phloem with some sieve tubes. The absence of sieve tubes in some samples might indicate that sieve tubes are dissolved as the culm grows older. The study further showed the position of phloem which is usually located in the direction of the peripheral region, while protoxylem vessels are found away from the peripheral region of bamboo. Therefore the position of the metaxylem and protoxylem vessels can give a clue to identifying the outer and inner parts of bamboo at the transverse section especially when the phloem is difficult to identify. SEM micrographs revealed sculptured arrangement of wood cells in a three-dimensioned perspective and thick-walled parenchyma cells different from micrographs from a light microscope. Photomicrographs were taken using a DC-12DX digital camera mounted on a Navite XSZ-20 Series light microscope at 80×. For SEM, observations of sections were carried out using a Jeol JSM 6390LV SEM at 15 kV and EDX, Nora system six. Anatomical descriptions were based on International Association Wood Anatomists (IAWA) codes.
Keywords: Fibre - SEM - Protoxylem - Phloem - Parenchyma - Tissues.

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INTRODUCTION

Bamboo (*Bambusa vulgaris* Schrad. ex J.C.Wendl.) has been a source of innumerable uses to man for ages. It is well known for its fast growth and excellent mechanical performance (Wang *et al.* 2014). The potential to produce long fibre pulp from some non-woods, woody grass and agricultural products has prompted and intensified bamboo research in recent years. Bamboo is an increasingly necessary economic asset in poverty eradication, economic and environmental development (FAO 2005). It is also an ancient woody grass widely distributed in tropical, subtropical and mild temperate zones, the species of bamboo that grow in Nigerian include *Bambusa vulgaris, B. arundinacea* (Retz.) Willd., *B. tulda* Roxb., *Dendrocalamus giganteus* Munro and *Oxytenanthera* spp. (RMRDC 1996).

Bamboo has been found to be as strong as timber and some species even exceed the strongest timbers in strength (ICDRC 1991), at the same time it is remarkably light, circular and strong, as a matter of fact, panel products made from bamboo were reported to have advantages of large size, and parallel and perpendicular strength properties that can be adjusted according to different demands (Zhang 2001). *B. vulgaris* lacks secondary thickening as opposed to what is obtained in trees, but possesses numerous vascular bundles

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consisting of xylem, phloem and fibre caps which are embedded in parenchymatous tissues. The sclerenchymatous fibres are the main stiffening elements providing mechanical support for the stem. This is because as the bamboo ages and grows taller, new cell wall layers are formed in the supporting fibres resulting in increased cell wall thickness (Gritsch *et al.* 2004) and in turn leads to a multilayered wall structure of the supporting fibres. This multilayered wall structure is characterized by an alternation of thick layers with low microfibril angles and thin layers with high microfibril angles (Crow & Murphy 2000).

Contrary to common intuition, bamboo is a very dense wood, its density runs two to three times that of pine and usually is more dense than oak (www.naveaching.com/shaku/structure.html.). Past work on *Bambusa blumeana* and *Gigantochloa laevis* (species of bamboo) by Espiloy (1987) revealed that there was a correlation between its anatomical characteristics (fibro-vascular bundle frequency, dimension of fibre and vessel) and its relative density, phsico-mechanical properties, shrinkage, moisture content, static bending and compression parallel to the grain. This type of correlation was further confirmed with age and location where the samples are taken (Wahab 2009). These signify that anatomy and physical properties of bamboo culms have significant effects on their durability and strength (Liese 1985, Latif & Tamizi 1993). This study intends to increase the database of information of bamboo by revealing the ultrastructures using an SEM. Of course, many notable research studies have been carried out on anatomical features of some hardwood species in Nigeria, ranging from micro-morphological features to both qualitative and quantitative analyses of some known hardwood species (Gill & Onuja 1984, Okoegwale & Gill 1990, Ogunkunle & Oladele 2008, Jayeola *et al*. 2009, Ogunwusi 2012, Adeniyi *et al*. 2013, Olatunji 2014). Several of these studies (which are commendable) identifying wood tissues and cells were often concluded at light microscopy, and as a result, more detailed ultra-structure reflecting the nature of inter-vascular pits in these tropical hardwood species could not be ascertained. This was so because the distribution of these features can hardly be observed under the light microscope when pits are minute (<4 μm) and/or when vestures are weakly developed (Jansen *et al.* 2004). This is of great concern when describing some Nigerian hardwood species only on the basis of light microscope, whereas all modern scientists studying wood classification and identification must do so through the use of advanced methods such as scanning electron microscopes (SEM), Transmission electron microscope or X-ray. Many specimens in the subtribe Dialianae appeared vestured when viewed under the light microscope (Quirk & Miller 1985), but SEM observation clearly showed that they lacked vestures. It could be therefore instructive to examine Nigerian Bamboo using an electron microscope as this will complement the light microscope study of the woody plant.

MATERIALS AND METHODS

Light Microscopy

Mature stems of *Bambusa vulgaris* were fell from the forest zone of Nigeria, and the herbarium samples were identified at the Forest Research Institute of Nigeria. Samples from stems were prepared into the smaller prismatic shape of 1 cm^3. Wood samples from the bamboo were not boiled in order to maintain the natural state of the specimens. Sectioning of wood materials was performed using a microtome sliding machine (Reichert, Vienna, Austria); the sections were washed with distilled water, covered with safranin and later washed with distilled water until the water became colourless. Dehydration was done by passing the wood sections through a series of bath of increasing concentrations of ethanol which replaced water. Sections were later covered with coconut oil (Adeniyi *et al*. 2016) for 1 hour and placed on a clean slide where a slight amount of Canada balsam was added as a mountant. The slides were examined under a Zeiss microscope (Standard 25) ×80 magnification, attached to a digital camera (SVP DC-12DX).

Scanning electron microscopy

Small blocks (3 mm^2) were attached to stubs using electron-conductive carbon paste. The samples were sputter-coated for 3 min with gold (Spi-Supplies, West Chester, PA, USA). Observations of the radial and tangential sections were carried out using a Jeol JSM 6390LV SEM (Jeol Ltd, Tokyo, Japan) at 15 kV and EDX, Nora system six.

RESULTS AND DISCUSSION

The results showed a cross-section from the culm of *Bambusa vulgaris* revealing a ground tissue of parenchyma cells that were around the vascular bundles; among the vascular bundles were fibre bundles which were made up of closely-packed scelerechymatous individual fibres that surrounded the conducting elements. Essentially, each vascular bundle is composed of a protoxylem vessel, two metaxylem vessels and phloem with some sieve tubes alongside the companion cells (Fig. 1A & B). The presence of sieve tubes was revealed in figure 1B where phloem was indicated. The presence of sieve tubes in bamboo had been reported by Liese (1987), but the absence of these sieve tubes as presented in figure 1A & C might indicate that sieve tubes and the accompanying companion cells could transform to form the third big pore usually positioned opposite the protoxylem vessel (pore) within the vascular bundle. Probably the sieve tubes are dissolved as the culm grows older. Hence, the third big pore in the vascular bundle is the position of the phloem which is usually located in the direction of the peripheral region. Protoxylem vessels are usually away from the peripheral region of bamboo, therefore the position of the metaxylem and protoxylem vessels can give a clue to identifying the outer and inner parts of bamboo at the transverse section especially when the phloem is difficult to identify. Vascular bundles in bamboo were more abundant toward the outer wall (Fig. 1C), *i.e.*, the proportion of vascular bundles continued to change from inner region to the periphery (Mustafa *et al.* 2011). The innermost part is a zone of the procumbent parenchyma cells (Fig. 1B); this zone is devoid of vascular bundles. It is essentially a soft tissue zone consisting of procumbent parenchyma cells as axial parenchyma cells are also rare in the zone. This may explain why bamboo is tougher at outer region than the innermost region; thus, from the outermost surface inwardly, the fibre structure, density and strength of bamboo might vary enormously; the outer layer may also have significantly higher specific gravity and bending properties than the inner layer owing to the number of fibres in the outer surface (Areghan *et al.* 2013).

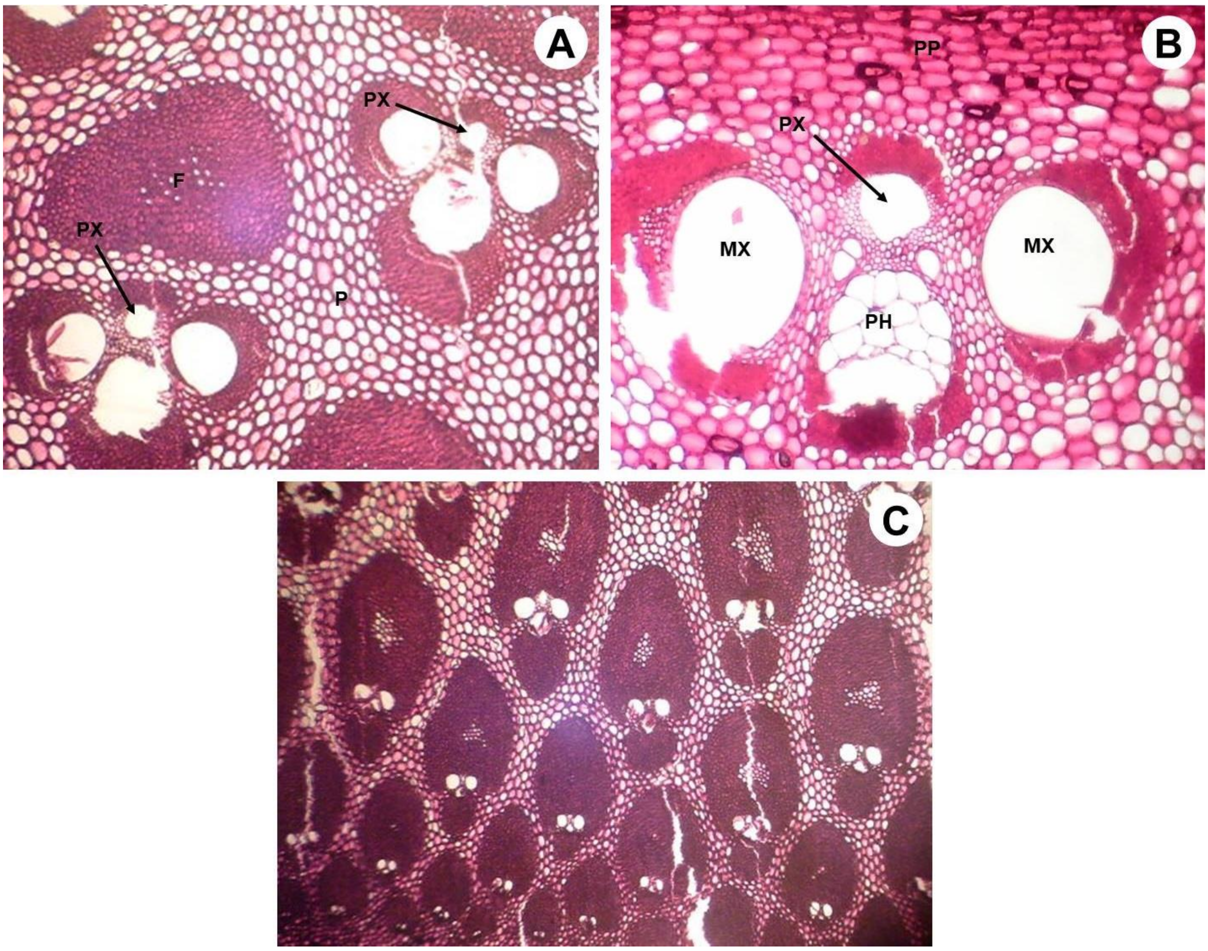

Figure 1. Transverse sections of *Bambusa vulgaris* Schrad. ex J.C.Wendl. under light microscope: **A**, Positions of fibre bundle (F), parenchyma cells (P) and arrowed showing protoxylem (PX); **B**, Positions of procumbent parenchyma cells (PP), metaxylem (MX), phloem with sieve tubes and companion cells (PH) and arrowed showing protoxylem (PX); **C**, Vascular bundles are more and smaller at outer region.

SEM micrographs revealed sculptured arrangement of wood cells in a three-dimensioned perspective (Fig. 2A) different from micrographs from a light microscope. The coarse, open-cell structure and rays so characteristic of various wood species were completely absent in *Bambusa vulgaris*. But there seemed to be revealed some thick-walled parenchyma cells (Fig. 2B) and some thin-walled, narrow, cylindrical and elongated axial parenchyma cells of several millimeters in length (possibly spanning the entire length of the plant) with numerous pits (Fig. 2C). Liese & Weiner (1996) had earlier identified long parenchyma cells that were characterised by thickened, polylamellate and lignified walls, and are considered as a reservoir where energy is stored in form of starch (Fig. 2B). The presence of thick-walled parenchyma cells in *Bambusa vulgaris* suggests that the nature of the cell wall of the ground tissue (parenchyma) contributes to the strength of the wood. Moreover, vessels in bamboo had simple pits that were slit-like (Fig. 2A); the fibres were thick-walled and of various diameters as seen in figure 2D. This might indicate that the structure of the fibres differs among the sheaths; a feature that also characterized the various sizes of rectangular parenchyma cells.

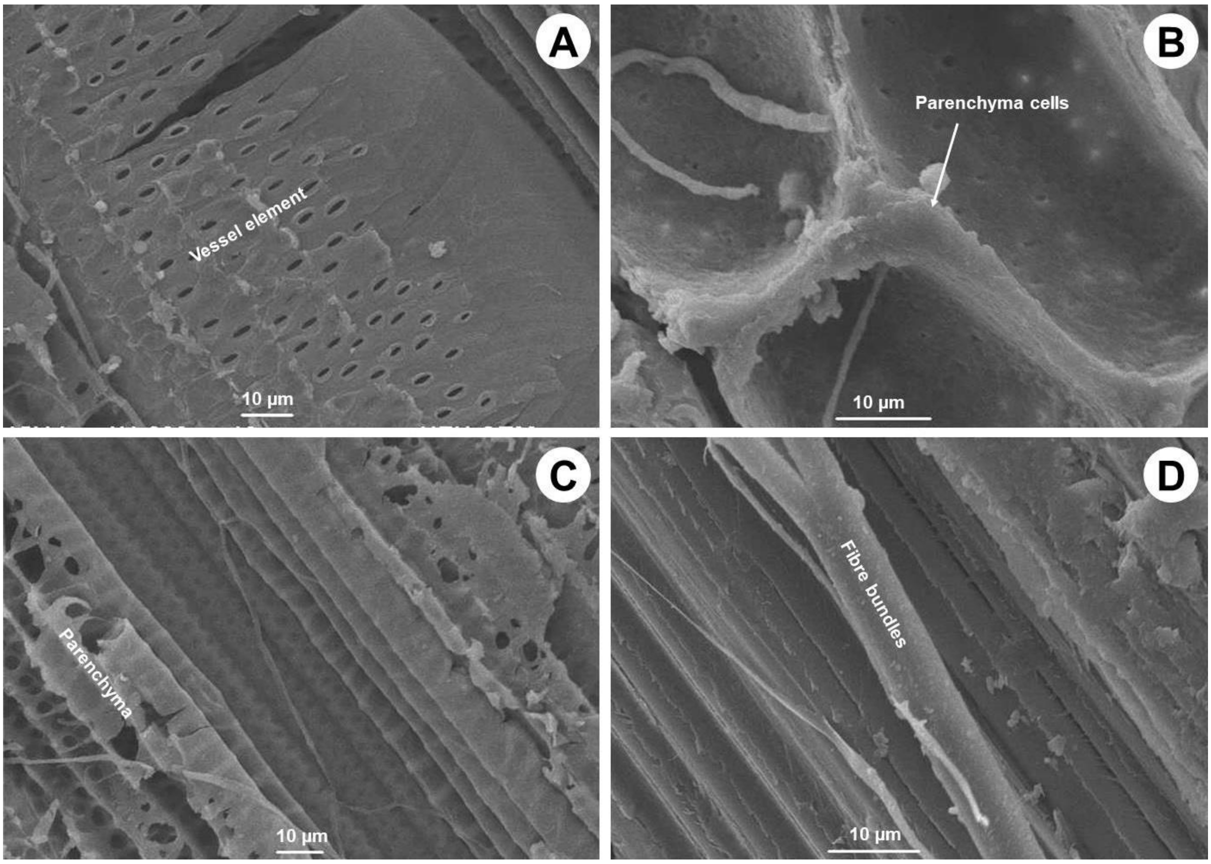

Figure 2. A, Vessel element with pits; **B**, Thick-walled parenchyma cells; **C**, Thin-walled parenchyma cells with numerous pits; **D**, Region of fibre bundles.

CONCLUSION

This study has shown the ultrastructures of *Bambusa vulgaris* and has brought to fore the features that are within the wood. Bambusa vulgaris actually is composed of metaxylem vessels, a protoxylem vessels, phloem, fibres and different types of parenchyma cells. Parenchyma cells in bamboo are both thin-walled and thick-walled. Protoxylem is towards the cavity (lacuna), while metaxylem and phloem are positioned towards the peripheral or outer region of the culm. This study has also revealed that the phloem can be devoid of the sieve tubes. Moreover, SEM micrographs revealed thick-walled parenchyma cells, an indication of the fact that sclerenchymatous fibres are not the only main stiffening elements providing mechanical support for the stem. However further investigations may be needed especially to reveal reasons behind the emptiness of some phloem tissues in the vascular bundle in which sieve tubes were absent.

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