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Microscopic Examination of Nodal Cell Wall Tissues from *Bambusa vulgaris* Schrad of Different Age Groups in a Rainforest Region, Nigeria

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Abstract
Studies on bamboo culms are currently increasing in intensity and scale with most of them being on their internodes, although, limited number of these studies showed that the nodes can also be useful. This research was therefore carried out to examine the microscopic features of cell wall structure of a bamboo's nodal tissues. Consequently, culms of *Bambusa vulgaris* Schrad of between ages of <1 and 3 years old were harvested from Ogba Biological Garden and Park and within the University of Benin campus, Benin City, Nigeria, sectioned into basal, middle and upper portions, taken to the laboratory and fixed in concentrated ethanol. Blocks of tissues of approximately 0.5 x 0.5 x 1.0 cm^3 were obtained from each of the nodes from the three sections and further immersed in increasing concentrations of ethanol for periods ranging from 1 to 3 hours. These tissues were also immersed in two different xylene solutions and later followed by the introduction of wax into the tissues. Using a microtome, thin slices of tissues of 30μm thickness were obtained from the blocks in the longitudinal, tangential and radial planes, stained with haematoxylin/eosin, mounted on glass slides and dried. Observation of cell wall tissues was carried out using a photomicroscope. Analyses of all the photomicrographs revealed that the nodal tissues appeared to contain comparatively larger vascular bundles at the base of all the culms, with those from the radial planes appearing to give more of this photo-impression. The size of the vascular bundles appeared to also decline from the base towards upper part in the three age groups. The vascular bundles were also observed to be larger in all the planes for older culms than the younger ones. However, it appeared that the frequency of the vascular bundles increased from the basal portion towards the upper in all the age groups. The outcomes of this research indicate that nodes may be employed in some applications for maximal utilisation of culms with more studies necessary to improve on these potentials.

Keywords: Bamboo nodes, lignocellulosic tissues, microscopic photo-impressions, longitudinal/transverse cross-sections

Introduction

Outcomes of studies carried out to add value to bamboo resource have assisted in increasing the awareness on their several applications and socio-economic benefits in certain tropical countries located in Asia and South America. This is in no way exempting other similar research carried out in Europe and North America on some bamboo species (Kumar *et al*., 1994; Gielis, 2002; Liese and Kumar, 2003; Liese, 2004; Chand *et al*., 2006; Nordin *et al*., 2007). Bamboo is a renewable natural resource also available in many African countries but with limitations in value addition owing to paucity of such research highlighted earlier (Erakhrumen, 2010). Nonetheless, the available published results of studies on bamboo in this part of the world have shown that they possess inherent potentials for more value-added utilisation (Erakhrumen, 2009; Erakhrumen, 2014).

As laudable and relatively informative as most of the research outcomes were, they are still limited in scope owing partly to the inadequate research facilities. Apart from this challenge, many of the studies, particularly those that have to do with cell wall and physico-mechanical properties of bamboo culm tissues, have focussed mainly on the internodes irrespective of the fact that the nodal portion also posses valuable characteristics that can be exploited for productive applications. It is almost customary for the nodal portion of bamboo culms to be removed whenever strength and/or mechanical tests such as tensile and static bending are to be carried out even as outcomes of studies such as Shao *et al*., (2010) has shown that presence of nodes did not have negative effects on tensile strength, bending strength and compressive strength parallel or transverse to bamboo grain.

Therefore, more studies that will assist in the efforts at maximal utilisation of the nodal portion of bamboo culms are necessary especially on those bamboo species from this part of the world, where such studies are scarce, as they are not unlikely to possess some characteristics dissimilar to and better than those reported from other parts of the world. Continuous investigation of cell wall properties of bamboo stems is important since these cells stay alive and are modified during the entire plant development, as unlike woody species, they develop only a primary shoot without secondary thickening growth which impedes geometrical adaptations and increases the necessity for structural optimisation at the material level (Wang *et al*., 2012a). Research on nodal portions of bamboo is necessary as they appear at intervals of about 10cm along the stem and are very vital for hardness and stability of the slender culms including conduction of water (Shao *et al*., 2010).

Investigations on these nodes are also important partly because a connection between the longitudinal and cross direction is made by the crossing walls of the nodes which consist of a sheath scar, nodal ridge, diaphragm and intranode between nodal ridge and sheath scar (Liese, 1987; Abd. Latif and Mohd. Tamizi, 1992). In addition, the fibres, being the main component of lignocellulosic cell walls, determine the mechanical properties of bamboo owing to their unidirectional arrangement in the tissue as well as their unique cell wall structure (Murphy and Alvin, 1992; Gritsch *et al*., 2004; Shao *et al*., 2010). Therefore, this research was carried out to investigate the cell wall structure of tissues from nodes obtained from the culms axially in three different age groups of bamboo.

Materials and Methods

Source of Bamboo Culms

Bamboo culms for the study were obtained from two locations within the same ecological zone from May to June, 2016. These locations were Ogba Biological Garden and Park and within the University of Benin campus, both located on the southern part of Edo State, Nigeria, lying between latitude 06°15´N–06°27´N and longitude 05°30´E–05°40´E. The topography is flat with gentle slope. The area has an annual rainfall of between 1,500 and 2,000mm with an average temperature of 25°C in the rainy season and 28°C in the dry season. It is part of a low lying plain covered with porous sand that rises gently north–eastward, with soils derived from sand stones and shades and very recent deposits susceptible to leaching (Egbe *et al*., 1989; Kalu and Anigbere, 2011).

Specimen Preparation and Microscopic Analyses

The different age groups to which the identified bamboo culms belong were determined before harvesting them from the wild using the methods described by Wahed Khan, (1962) and Banik, (1988). The harvested bamboo culms from three different age groups (<1 year, 1-2 years and 2-3 years) were longitudinally sectioned into three so that nodes were obtained from the basal, middle and upper sections of the culms. Specimens' blocks of the nodal wall measuring approximately 0.5 x 0.5 x 1.0 cm^3 were prepared in line with simple modifications to that of Gritsch *et al*., (2004). The blocks of tissues were obtained from the already sectioned culms that had been earlier fixed in concentrated ethanol on the field to aid the process of tissue dehydration. The sampled blocks of tissues from the different nodes were further immersed in ethanol solutions of increasing concentrations from 70% until 100% water free alcohol was reached, for a period ranging from 1 to 3 hours, to ensure water in the tissue was gradually replaced by the alcohol.

Complete removal of moisture from these tissues is a necessary step before the embedding of wax into the tissues, since wax itself is hydrophobic. Gradual replacement of water in the sampled tissues by alcohol will aid in avoiding excessive distortion of the tissue, removal of water soluble proteins and dissolution of certain lipids. After dehydration, the tissues were immersed in two different xylene solutions so that alcohol can be gradually replaced by xylene to make the tissues more transparent and clearer. This was followed by total replacement of the xylene in the tissues with molten wax. Following this, using a Leica rotatory microtome, thin slices of tissues of 30µm thick were obtained from the blocks in the longitudinal, radial and tangential planes. These slices were stained afterwards with haematoxylin and eosin and mounted on glass slides with DPX medium and allowed to dry.

Observations of cell wall tissues were carried out using a microscope at X40 magnification and photomicroscope at X100 magnification to obtain the photomicrographs of the slides' content for comparison. The photomicrographs were obtained with the photomicroscope at the Histology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria.

Results

Plates 1 to 27 show the pictorial representations of the photomicrographs obtained from the microscopic examinations of the different lignocellulosic tissues obtained from all the sampled nodal portions. For bamboo culms with age less than a year old, plates 1, 2 and 3 are photomicrographs of nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, obtained from the basal culms portion while plates 4, 5 and 6 are for photomicrographs of nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the basal portion of the culms with ages ranging between 1 and 2 years old, with plates 7, 8 and 9 representing photomicrographs of nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, also at the basal portion of the culms with ages ranging between 2 and 3 years old.

In addition, for samples obtained from bamboo culms with ages ranging between 1 and 2 years old, plates 10, 11 and 12 represent the impressions captured as photomicrographs of nodal cell wall tissues sampled from the longitudinal, tangential and radial planes respectively, at the middle portion of the culms. Plates 13, 14 and 15 are the photomicrographs for nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the middle portion of the culms with ages ranging between 1 and 2 years old while the pictures captured in plates 16, 17 and 18 are the photomicrographs of nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the middle portion of the culms with ages ranging from 1 to 2 years old.

Furthermore, for bamboo culms with ages ranging from 2 to 3 years old, the photomicrographs depicted by plates 19, 20 and 21 are those obtained for nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the upper portion of the culms. The pictorial representation captured by plates 22, 23 and 24 are the photomicrographs obtained for nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the upper portion of the culms having ages ranging between 2 and 3 years old while plates 25, 26 and 27 represent the photomicrographs obtained for nodal cell wall tissues from the longitudinal, tangential and radial planes respectively, at the upper portion of the culms with their ages ranging from 2 to 3 years old.

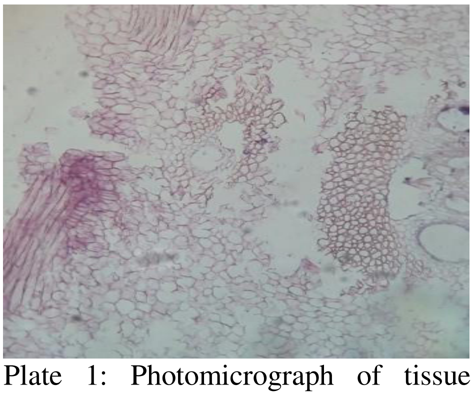

Plate 1: Photomicrograph of tissue obtained in the longitudinal plane from basal nodes with age less than 1 year old

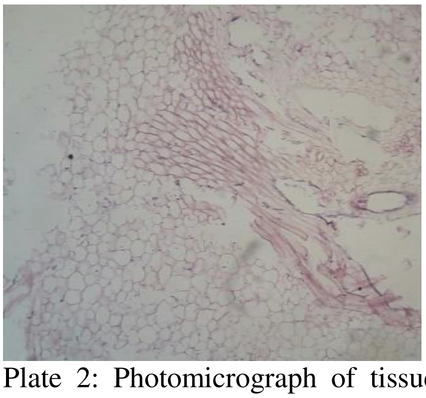

Plate 2: Photomicrograph of tissue obtained in the tangential plane from basal nodes with age less than 1 year old

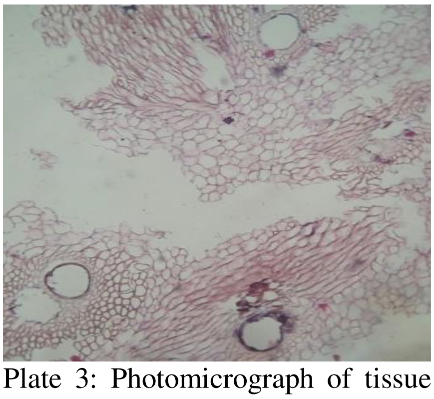

Plate 3: Photomicrograph of tissue obtained in the radial plane from basal nodes with age less than 1 year old

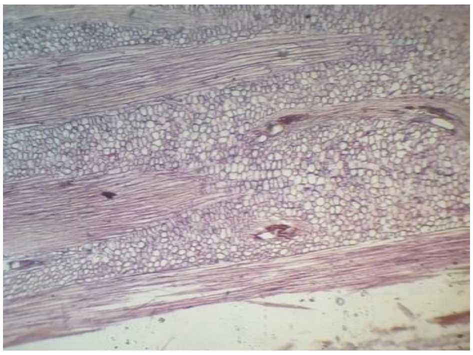

Plate 4: Photomicrograph of tissue obtained in the longitudinal plane from basal nodes with ages between 1-2 years old

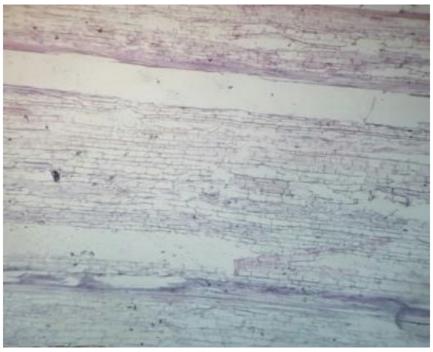

Plate 5: Photomicrograph of tissue obtained in the tangential plane from basal nodes with ages between 1-2 years old

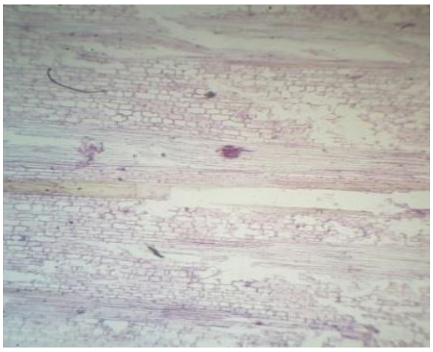

Plate 6: Photomicrograph of tissue obtained in the radial plane from basal nodes with ages between 1-2 years old

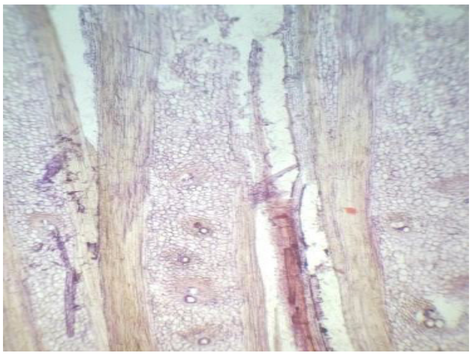

Plate 7: Photomicrograph of tissue obtained in the longitudinal plane from basal nodes with ages between 2-3 years old

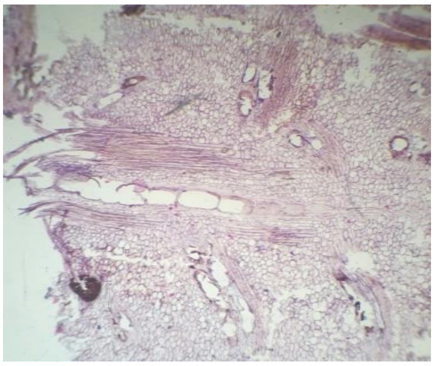

Plate 8: Photomicrograph of tissue obtained in the tangential plane from basal nodes with ages between 2-3 years old

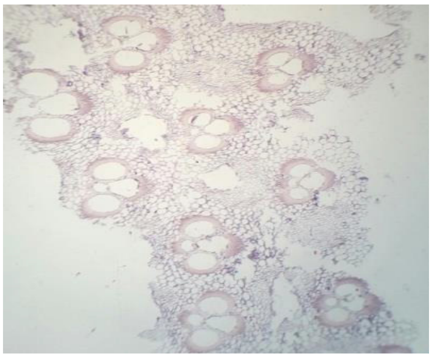

Plate 9: Photomicrograph of tissue obtained in the radial plane from basal nodes with ages between 2-3 years old

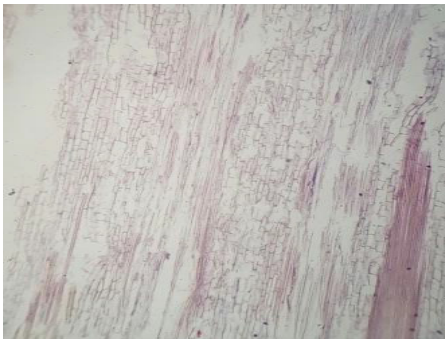

Plate 10: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the middle portion of culms with ages less than 1 year old

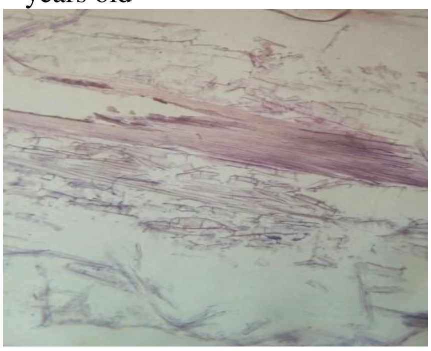

Plate 11: Photomicrograph of tissue obtained in the tangential plane from nodes in the middle portion of culms with ages less than 1 year old

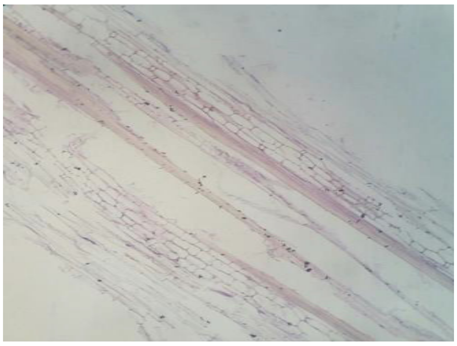

Plate 12: Photomicrograph of tissue obtained in the radial plane from nodes in the middle portion of culms with ages less than 1 year old

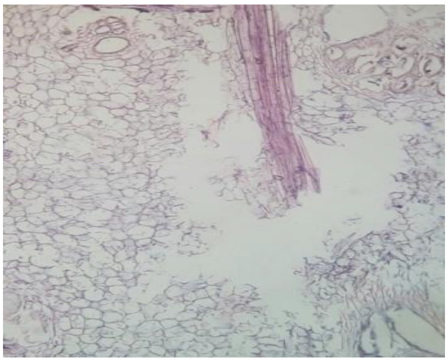

Plate 13: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the middle portion of culms with ages between 1 and 2 years old

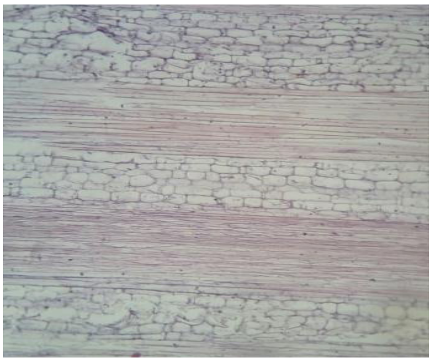

Plate 14: Photomicrograph of tissue obtained in the tangential plane from nodes in the middle portion of culms with ages between 1 and 2 years old

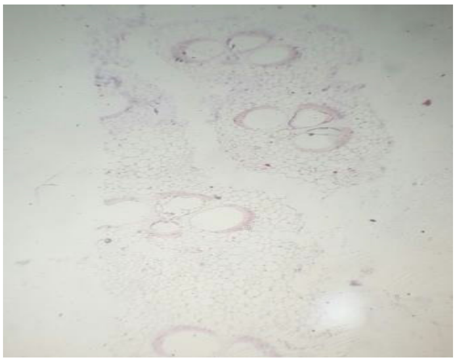

Plate 15: Photomicrograph of tissue obtained in the radial plane from nodes in the middle portion of culms with ages between 1 and 2 years old

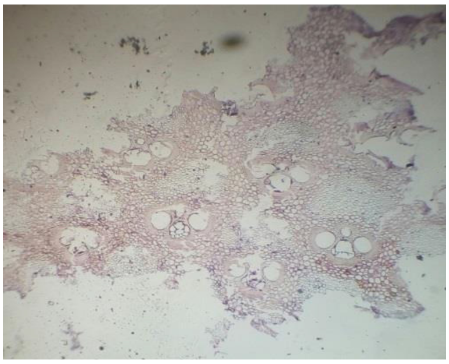

Plate 16: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the middle portion of culms with ages between 2 and 3 years old

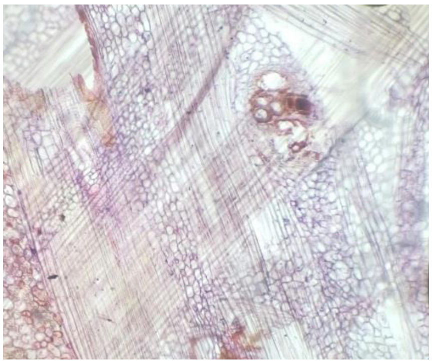

Plate 17: Photomicrograph of tissue obtained in the tangential plane from nodes in the middle portion of culms with ages between 2 and 3 years old

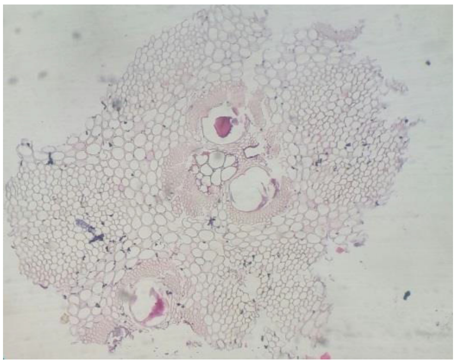

Plate 18: Photomicrograph of tissue obtained in the radial plane from nodes in the middle portion of culms with ages between 2 and 3 years old

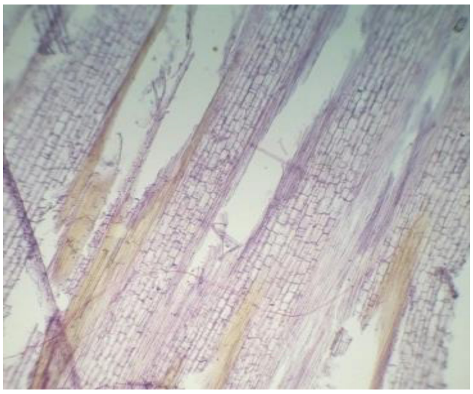

Plate 19: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the upper portion of culms with ages less than 1 year old

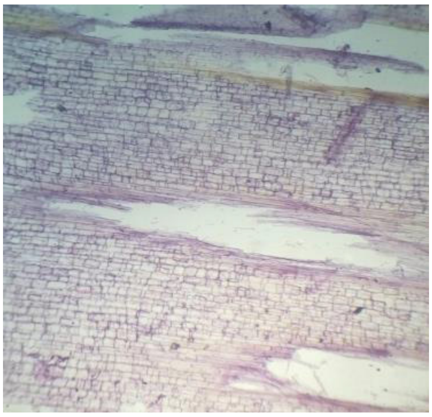

Plate 20: Photomicrograph of tissue obtained in the tangential plane from nodes in the upper portion of culms with ages less than 1 year old

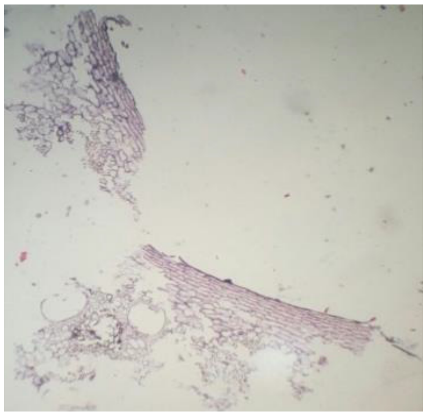

Plate 21: Photomicrograph of tissue obtained in the radial plane from nodes in the upper portion of culms with ages less than 1 year old

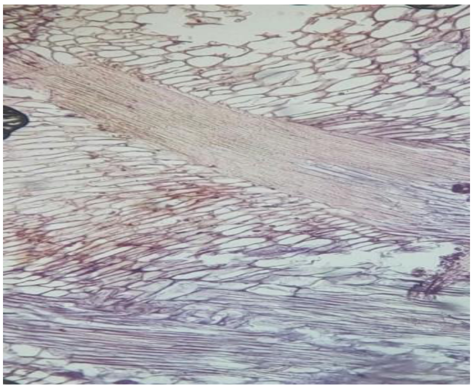

Plate 22: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the upper portion of culms with ages between 1 and 2 years old

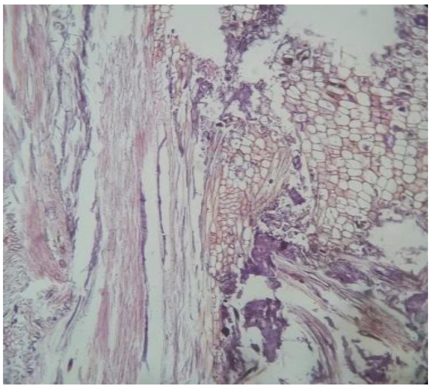

Plate 23: Photomicrograph of tissue obtained in the tangential plane from nodes in the upper portion of culms with ages between 1 and 2 years old

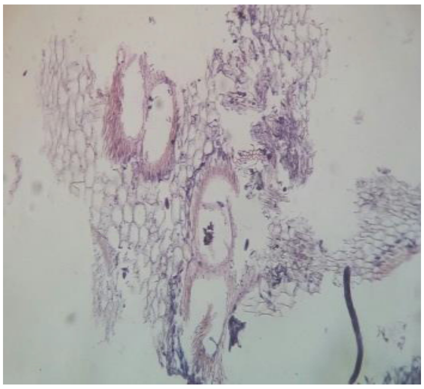

Plate 24: Photomicrograph of tissue obtained in the radial plane from nodes in the upper portion of culms with ages between 1 and 2 years old

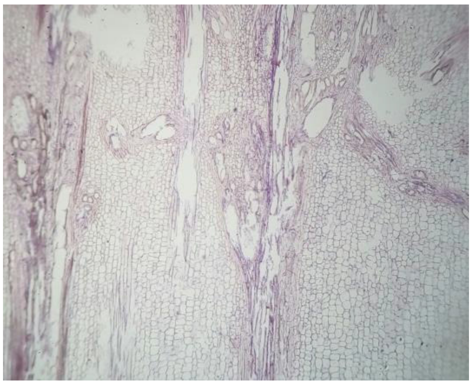

Plate 25: Photomicrograph of tissue obtained in the longitudinal plane from nodes in the upper portion of culms with ages between 2 and 3 years old

Plate 26: Photomicrograph of tissue obtained in the tangential plane from nodes in the upper portion of culms with ages between 2 and 3 years old

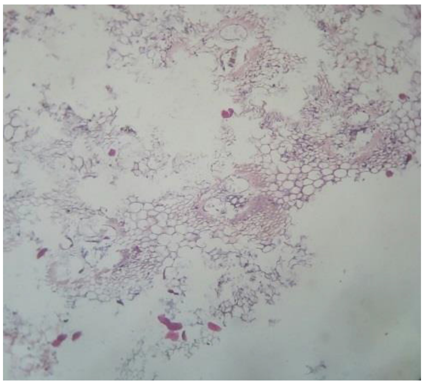

Plate 27: Photomicrograph of tissue obtained in the radial plane from nodes in the upper portion of culms with ages between 2 and 3 years old

Discussion

The various types of cells in bamboo's lignocellulosic tissues can be summarily classified as parenchymatous ground tissues and sclerenchymatous tissue made up of vascular bundles. The ground tissue and bundle sheaths account for about 55 and 40% respectively of the whole bamboo tissue while the rest are vessel and primary xylem, with bamboo cells between two nodes strictly oriented axially (Liese, 1987; Abd. Latif and Mohd. Tamizi, 1992; Shao *et al*., 2010). Analyses of all the photomicrographs obtained from the nodal tissues sampled from the longitudinal, tangential and radial planes revealed that the tissues appeared to contain comparatively larger vascular bundles at the basal part of all the culms than the middle and upper parts, with the samples of tissues from the radial plane appearing to give more of this photo-impression than those from the other planes.

It is noteworthy that the vascular bundle is oval in shape and varies in size depending on the location/position as well as species. Sclerenchyma sheaths surround the xylem and phloem, and are known to be principal supporting tissues within the vascular bundles with the microstructures being similar at both the internodal and nodal sections (Grosser and Liese, 1971; Bhat *et al*., 2011). Observation of the photomicrographs obtained for nodal tissues in both the tangential and radial planes revealed a scattered vascular bundles within ground tissue composed of parenchyma cells as also observed by Gritsch *et al*., (2004), although, studies showed that bamboo fibre and parenchyma cells possess typically multilayered or polylamellated cell walls with alternating transverse and oblique cellulose microfibril angles (Tono and Ono, 1962; Parameswaran and Liese, 1980).

The size of the vascular bundles, as depicted by the photomicrographs, declined along the culms from the basal portions towards the upper portions. This pattern was observed to be consistent in the three different bamboo age groups. This observed trend appeared to be similar to some others in literature such as Grosser and Liese, (1971), Kumar and Dobriyal, (1992), Abd. Latif *et al*., (1993), Kelemwork, (2009), Santhoshkumar and Bhat, (2015) with tapering of bamboo stems being the likely cause of smaller vascular bundles at the upper portions of the culms (Abd. Latif and Mohd. Tamizi, 1992). The sizes of the vascular bundles were also observed to be larger in the tissues obtained from the different planes along the culms in older bamboo stems when compared to those from younger stems. This is in line with other recent similar studies such as Huang *et al*., (2015).

Characterising the structure of bamboo's lignocellulosic cell wall is necessary since many of its fibre characteristics like cell wall thickness, lumen diameter, number of cell wall layers and lignifications of cell wall have been demonstrated to vary according to their location in the culm and within vascular bundles, as well as with state of maturation of the culms (Murphy and Alvin, 1992; Abd. Latif and Liese, 2001; Bhat, 2003; Wang *et al*., 2012b). Thus, these variations in culms characteristics have been proven, through researches, to influence many of the applications to which bamboo culms are put. For instance, bamboo's durability, toughness, workability and strength are associated with culms' anatomical properties which are the basis for understanding their physical, mechanical and other utilisation potentials (Espiloy, 1992; Kelemwork, 2009; Wang *et al*., 2012b).

These photo-impressions obtained from the photomicrographs that the nodal cell wall appeared to have larger vascular bundles at the basal than the middle and upper parts and likewise in nodes obtained from older culms compared with younger ones may imply that when tissues from nodes are to be used in some applications that has to do with mechanical support, it may be assumed that tissues from basal parts particularly from older culms will be more relevant for this purpose. Nevertheless, other factors, such as frequency of vascular bundles in the different tissues along the culms and among the different age groups should also be considered. For instance, some studies have shown that frequency of vascular bundles increased from the basal portion towards the upper portion leading to increase density at this upper part with size of vascular bundles not being directly correlated with age (Abd. Latif and Mohd. Tamizi, 1992; Santhoshkumar and Bhat, 2015).

Irrespective of some of these variations axially along the culms in some of the published studies, some mechanical properties like bending strength, shear and compressive strength parallel to grain and compressive strength perpendicular to grain had been observed to be high in the nodal area owing to the high proportion of fibres in this area (Shao *et al*., 2010). This could be a possible explanation for why density and shrinkage were significantly correlated to radial/tangential ratio of vascular bundles in some bamboo tissues by Abd. Latif *et al*., (1993) most especially as the microstructures of internodes and nodes are similar in the same species (Bhat *et al*., 2011). The dimensions and quantity of anatomical constituents characterising the nodal cell wall were not measured in this study but the results of microscopic observations appeared to be in line with some of the published data and trends in literature as had also been highlighted earlier above.

Conclusion and Recommendation

The results obtained from this research showed that samples of lignocellulosic tissues obtained from the different nodes along the stems of the various bamboo age groups have certain features, as shown by the different photomicrographs, that appeared similar to stem tissue material for this and some other bamboo species studied in similar manner in literature. Succinctly, it can be said that the microscopic view of the nodal cell wall tissues in this study point to the fact that nodes are likely not to be inferior for utilisation in applications requiring some of the characteristics highlighted earlier, thus, it is necessary that more research that will not only improve knowledge concerning this part of the culms but also those that will assist in adding value to it should be intensified.

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