KFRI Research Report No.216 ISSN 0970-8103

ANATOMICAL CHANGES ASSOCIATED WITH CULM MATURATION IN *BAMBUSA BAMBOS* (L.) Voss AND *DENDROCALAMUS STRICTUS* Nees

K. V. Bhat

Kerala Forest Research Institute
Peechi 680 653, Kerala, India

October 2001

KFRI Research Report No. 216

ANATOMICAL CHANGES ASSOCIATED WITH CULM MATURATION IN *BAMBUSA BAMBOS* (L.) Voss AND *DENDROCALAMUS STRICTUS* Nees

(FINAL REPORT OF THE PROJECT KFRI 309/98)
SEPTEMBER 1998 - AUGUST 2001

K. V. Bhat
Wood Science Division

Kerala Forest Research Institute
Peechi 680 653, Kerala, India

October 2001

ABSTRACT OF THE PROJECT PROPOSAL

1. Project No. : KFRI 309/98

2. Title of the Project : Anatomical changes associated with culm maturation in *Bambusa bambos* (L.)Voss and *Dendrocalamus strictus* Nees

3. Objectives :
   1. To document the changes in culm internodal structure of *B. bambos* and *D. strictus* in relation to increasing culm age
   2. To identify changes in external morphology of culms at corresponding stages of maturation
   3. To analyse the status of storage metabolites within culm tissue at various stages of maturation

4. Date of commencement : September, 1998

5. Date of completion : August, 2001

6. Funding agency : Kerala Forest Department (Dev.)

7. Project team
   Principal investigator : K. V. Bhat

8. Duration of study : 3 years

CONTENTS

Abstract	1
Introduction	2
Review of Literature	3
Materials and Methods	4
Results and Discussion	5
Structural variation within and between culm internodes	5
Structural changes during culm maturation	8
Age-related changes in physical properties	10
Storage metabolites in culm tissues	12
Morphological changes in culms with increasing age	13
Conclusions	13
Acknowledgements	14
References	14

ABSTRACT

The anatomical changes occurring during the maturation of culms were investigated in two common bamboo species *Bambusa bambos* (L.) Voss and *Dendrocalamus strictus* Nees by carrying out a comparative study of culm internodal material of different ages starting from 2 months up to 60 months. The culm wall consisted of ground parenchyma tissue enclosing a large number of fibro-vascular strands (bundles) that varied in their size, structure and relative proportion in different parts of the culm both in axial and radial directions. The outer, denser part of the culm wall had smaller but more numerous bundles and denser fibrous tissue as compared to the inner part, which contained more of soft tissues. The main change that occurred during culm maturation was the thickening of cell walls and lignification. Cell wall thickening and lignification progressed from outer to inner parts of the culm wall, and from culm base towards top. Within a fibro-vascular strand, these changes first occurred in fibres contiguous to the vascular tissues and then progressed to outer parts of the strand. In peripheral fibrous strands, the outermost fibres matured first followed by inner ones. Cell wall thickening of fibres was accomplished by addition of lamellae leading to polylamellate cell wall structure. In ground parenchyma, although the wall thickening was evident, lamellation was not distinct. These changes in cell wall structure led to increase in basic density of the culm material. The increase in density was dramatic during the first two years in both *B. bambos* and *D. strictus* which subsequently became more gradual or stable suggesting a culm maturation period of about two years in both the species. The moisture content percentage, which showed an inverse relationship with density, declined rapidly during the first two years and reached a stable value in later years. Storage metabolites in culm tissues consisted mainly of starch; lipids were not found, and proteins which were conspicuous in young material, decreased with culm age. Storage starch, which is generally believed to increase with culm age, did not show any direct relation with culm age in the present study. It was also found that from the external morphological changes accompanying culm maturation, it was possible to judge only the approximate age of the culms.

INTRODUCTION

Besides being used in housing and agricultural sectors traditionally for centuries, bamboo has been a popular raw material for pulping, bamboo-board and cottage industries. This resource has an important place in the economy of Kerala and other states of the country due to its contribution to industry, rural employment, agriculture, and housing. In rural areas, bamboo is used in construction even today as a cheaper substitute to timber; thus it is aptly nicknamed as 'poor man's timber'. In Kerala, besides forest lands, homesteads and farmlands are the major sources of bamboo supply. Although about 27 species of bamboos are known to occur in the State, the most common ones are *Bambusa bambos* (L.) Voss and *Dendrocalamus strictus* Nees. A significant proportion of rural population in Kerala is engaged in bamboo extraction and traditional bamboo-craft industry.

Bamboo is a highly versatile material having multiple end-uses like construction, pulping, basketry, mat-weaving, board manufacture, etc. The raw material quality requirements for these diverse end-uses are quite different. However, due to their wide range of variation in physical, mechanical and processing characteristics, bamboos are able to meet these requirements. The properties of bamboos vary not only between species, but also within a species depending on growth factors, maturity status of culms or their age. Since the fibro-vascular tissue of culms undergoes continuous changes with increasing culm age, harvesting the culms at appropriate stages of their maturity to suit the particular end-use has great importance in its utilization. For example, mature or over-mature material may be ideal in meeting the highest strength requirements of constructional uses, but it may not be a suitable material for slivering or mat-weaving since the fibre becomes brittle with age. Although experienced bamboo workers are able to select material of appropriate maturity to suit the end-use, the scientific basis of the practice is incompletely understood.

Another important consideration relevant to utilization of bamboo is its perishability. Bamboo culms are prone to attack by insect borers and are damaged quickly in storage or service. The borer problem is more severe than is generally expected; in open storage yards, the material is often reduced to powdery residue in less than six months. Even under partly protected conditions such as in a roof frame it is degraded in less than a year's time. It is often found that bamboo harvested during certain periods of the year or month is able to evade borer attack; thus farmers or experienced bamboo cutters give attention to timing their harvesting operation. However, this phenomenon of evasion of borer damage has not been scientifically validated. Susceptibility of the bamboo material to insect borers is generally correlated with reserve carbohydrates, mainly starch, stored in culm tissues. The presence or absence of starch in tissues is found related to culm age and/or the season of harvesting.

It is obvious that our knowledge of culm maturation process and storage metabolism in bamboos is inadequate to explain these age-related changes in structure and composition of culm tissues. In this regard, anatomical techniques offer a possibility of depicting the cellular and subcellular changes associated with the process. Such studies are important not only for a deeper understanding of the raw material properties for utilization, but also useful for developing appropriate harvesting practices for optimum use of resources.

REVIEW OF LITERATURE

Although bamboo was recognized as an important resource during the early part of nineteenth century, research on anatomical structure and fibre characteristics of bamboos gained a momentum only since the seventies. Early studies on bamboo anatomy were mostly related to fibre characteristics (Shigematsu, 1940; Preston and Singh, 1950; Tamolang *et al.*, 1958; Ghosh and Negi, 1959, 1961; Pattanath, 1972;) or structure of culms with reference to systematics (Velasquez and Santos, 1931; Ota and Sugi, 1953; Metcalfe, 1960; Ghosh and Negi, 1960; Lee and Chin, 1960; Pattanath and Ramesh Rao, 1969). Subsequently, as the potential of bamboo for multiple end-uses was recognized anatomical research extended to new frontier areas such as structure-property correlations, structural variation, cell wall ultrastructure, culm maturation and ageing, and processing properties. Besides, several valuable contributions (Grosser and Liese, 1971, 1973; Grosser and Zamuco, 1973; Liese, 1980; Jiang and Li, 1983; Rong, 1985; Agrawal and Luxmi Chauhan, 1990; Qiao, 1991; Jiang, 1992; Wang *et al.*, 1994; Sekar and Balasubramanian,1994a,b,c; Muller, 1996) have resulted from a continued effort to develop anatomical techniques for identification and classification of bamboos.

Since most of the properties of bamboo are dependent on its anatomical structure, considerable attention has been given to investigate this inter-relationship. The physical, and mechanical properties of bamboo were found correlated to structure and composition of culm tissue (Janssen, 1981; Espiloy, 1987, 1992, 1994; Liese, 1987a, b; Widjaja and Risyad, 1987; Abd. Latif *et al.*, 1990; Abd. Latif and Mohd Zin, 1992; Sattar *et al.*, 1994; Zhang *et al.*, 1995). Studies on ultrastructure of the cell and cell wall (Fujii, 1985; Parameswaran and Liese, 1975,1976,1977,1980; Liese, 1998; Tono and Ono, 1962; Wu and Leu, 1987) have helped to provide a further insight into the structure-property relationship. Similarly, the influence of anatomical structure on penetration of preservative chemicals during treatment has also been examined by some investigators (Younus-uzzaman, 1991; Wu *et al.*, 1992; Liese, 1997).

The structural changes taking place during maturation and ageing of bamboo culms have been examined in a few studies. It is found that the longitudinal growth of culm internodes is due to elongation of cells, mainly fibres, and is completed in a few days (Hsiung *et al.*, 1980). During the maturation phase that follows, there is a thickening and lignification of cell walls which proceeds at different rates across the radius of the culm wall (Alvin and Murphy, 1988; Majima *et al.*, 1991; Liese and Weiner, 1996, 1997; Murphy and Alvin, 1997a, b). Studies on ultrastructure of fibre walls have shown that the thickening of cell walls is due to deposition of additional lamellae. The process of fibre maturation is believed to prolong over many growing seasons (Liese, 1998). However, it has been observed in some species that lignification of culm tissues is completed within one growing season (Itoh, 1990). The cell wall thickening of fibres and parenchyma is found to continue even after maturation phase and is believed to be due to ageing (Alvin and Murphy, 1988; Liese, 1998).

The susceptibility of bamboo to insect borers, mainly beetles, can be regarded as the main limiting factor in its utilization. It is believed that the insect borers feed on the starch stored in culm tissues and the large variation in susceptibility of bamboos to borers is

attributed to difference in starch content with respect to species, culm age and harvesting season (Liese, 1998; Sulthoni, 1987; Alvin and Murphy, 1988; Weiner and Liese, 1996). However, a number of conclusions on biological resistance of the culms against insect borers are based on casual observations and not on scientific investigations.

MATERIALS AND METHODS

Culm internodal samples of *Bambusa bambos* and *Dendrocalamus strictus* were collected mostly from homesteads and also from forest areas of Kerala. Culms were selected so as to represent approximate age classes such as 2 months, 6 months, 12 months, 18 months, 24 months, 36 months, 48 months and 60 months. Culm age was recorded as known to the owners of the respective holdings and by counting the number of months lapsed after normal culm production in July-August. In total, 21 culms of *B. bambos* and 30 culms of *D. strictus* were used for the study. After felling the selected culms, 1 m long cuttings with 4 to 6 intact internodes were collected from the base, mid-height and top portions of each culm. Additionally, for moisture content determination, samples of 5 cm length were collected from each of the three representative portions of the culm and were enclosed in polythene bags to prevent moisture loss during transit. The culm diameter, lumen width and culm wall thickness were recorded. After reaching the laboratory, the cuttings were further subdivided into samples of suitable size for anatomical study and determination of basic density. The samples meant for microtomy were fixed in FAA and later transferred to 50% ethyl alcohol. Another set of samples were also preserved in 4% formaldehyde solution for histochemical testing of lipids. Basic density and moisture content percentage of the samples were determined gravimetrically following the conventional method of oven-drying the material. About 50 cm long left over culm segments were stored under laboratory condition for periodic observation on borer attack.

For anatomical study, 1 cm^3 blocks of culm internode were prepared and transverse and longitudinal sections of 20 μm thickness were cut on a Reichert sliding microtome. The sections were stained in Tannic acid-Ferric chloride and Safranin (Berlyn and Miksche, 1976), dehydrated and mounted in DPX. The following stains and histochemical reagents were used for localization of starch, lipids and proteins:

Iodine-Potassium Iodide (I_2KI)	- for starch (Johansen, 1940)
Sudan Black 'B'	- for lipids (Gomori, 1952)
Mercuric Bromophenol Blue	- for proteins (Berlyn and Miksche,1976)
Phloroglucinol-HCl	- for lignin (Johansen, 1940)

The abundance of vascular bundles was estimated by counting the bundles per cm^2 and tissue proportions across the culm wall were estimated by tracing the cross-sectional views on tracing film and calculating the area occupied by each tissue. Fibre dimensions such as length and double wall thickness were measured from macerated material. Maceration was done using a 1:1 mixture of hydrogen peroxide and glacial acetic acid.

Direct application of the reagent I_2KI to the longitudinal surface of the culm wall was adopted for quick verification of the presence or absence of starch in culm tissues. Culm samples, so tested, were stored for periodic observation on borer infestation.

RESULTS AND DISCUSSION

Structural variation within and between culm internodes

As common to majority of bamboos, the culm internodes of *Bambusa bambos* and *Dendrocalamus strictus* are divisible into a culm wall and a central cavity called lacuna. The epidermal and hypodermal layers of the culm wall constitute a narrow peripheral cortex while the major proportion of the culm wall is formed by strands of fibro-vascular tissue embedded in a matrix of ground tissue. The culm wall is lined on its inner side by layers of cells forming a pith ring. The morphology and distribution of fibro-vascular tissues vary not only between different species but also in different parts of a culm. A detailed knowledge of this variation is important for taxonomists as well as utilization technologists. Details of variation in anatomical structure within a culm have also been found to have great relevance in studies on maturation of culms. A number of studies have shown that the maturation process of fibres proceeds differently over the cross section of a culm and is influenced by their position within a fibro-vascular strand and position of the strand in the culm cross section (Alwin and Murphy, 1988; Majima *et al.*, 1991; Liese and Weiner, 1996, 1997; Murphy and Alwin, 1997a, b). From the present study also it was evident that cell wall thickening and lignification of tissues at any stage of maturity was variable in relation to their cross sectional position and height level within the culm. Therefore, a brief account of the structural variability observed in *Bambusa bambos* and *Dendrocalamus strictus* is given below.

A cross sectional view of the culm internode of *Bambusa bambos* and *Dendrocalamus strictus* exhibited the structural features of a monocot shoot. The peripheral portion of the culm wall consisted of a single layer of epidermis and a narrow homogeneous cortical region of thin walled parenchymatous cells while the major part of the culm wall consisted of a broad zone of ground tissue with numerous fibro-vascular strands. On the inner side facing the culm cavity, a pith ring was found which was 8-12 cell thick in *B. bambos* and 6-8 cell thick in *D. strictus*.

The size, structure and distribution of the vascular bundles across the culm wall showed a gradation from periphery inwards. The outer denser part of the culm wall had compactly arranged smaller bundles having massive fibrous sheaths and a lower proportion of vascular tissue. Towards inner side, there was a decrease in the proportion of fibrous tissue and an increase in the proportion of vascular tissues within the bundles. These changes were accompanied by a decrease in compactness or frequency of bundles per unit cross sectional area. The bundle size that increased from outer to inner parts of the culm wall had a little reduction towards the inner boundary. Average values of tissue proportions obtained for different portions of culm wall in *B. bambos* and *D. strictus* are given in Table 1.

From Table 1 it is evident that the proportion of fibrous tissue that imparts rigidity and hardness to the culm material decreases from culm periphery towards inner regions with a concomitant increase in softer ground tissue. Such variation common to most bamboos has been the main reason for the wide difference in physical and mechanical properties across the culm wall.

Table 1. Tissue proportion in different parts of culm wall in *B. bambos* and *D. strictus*

	Bambusa bambos			*Dendrocalamus strictus*		
	Outer part	Mid part	Inner part	Outer part	Mid part	Inner part
Fibrous tissue (%)	44.75 (9.58)	36.32 (5.55)	26.88 (5.75)	39.61 (8.73)	33.40 (10.3)	28.33 (6.36)
Vascular tissue (%)	5.18 (1.12)	6.37 (1.67)	8.51 (2.37)	6.76 (1.75)	6.87 (2.13)	7.50 (1.47)
Ground tissue (%)	50.09 (7.99)	57.31 (6.19)	64.61 (7.5)	53.42 (9.14)	59.74 (8.51)	64.17 (7.52)
No.of bundles per cm^2	283 (67)	111 (37)	116 (28)	379 (75)	201 (62)	148 (38)

- Figures in parentheses show SD of the mean

Form and size of vascular bundles are other features that varies widely across the culm wall. This variation has been intensively investigated in a number of bamboos for characterization of bamboo taxa (Grosser and Liese, 1971; Grosser and Zamuco, 1973; Wen and Chou, 1984; Wu and Hsieh, 1990; Wang *et al.*, 1994). Bundles in the peripheral part of the culm wall are generally smaller and more numerous and are rather crowded. These bundles are devoid of or with highly reduced vascular tissue and their fibre sheaths are often fused into one composite structure. Bundle structural types distinctive of the species occur at about halfway between outer and inner boundaries of the culm wall. In the inner part, vascular bundles are smaller and have greater proportion of vascular elements and reduced sheaths.

The variation in size, shape and distribution of vascular bundles found in culm cross sections of *B. bambos* and *D. strictus* is shown in Fig.1. At the periphery, the bundles

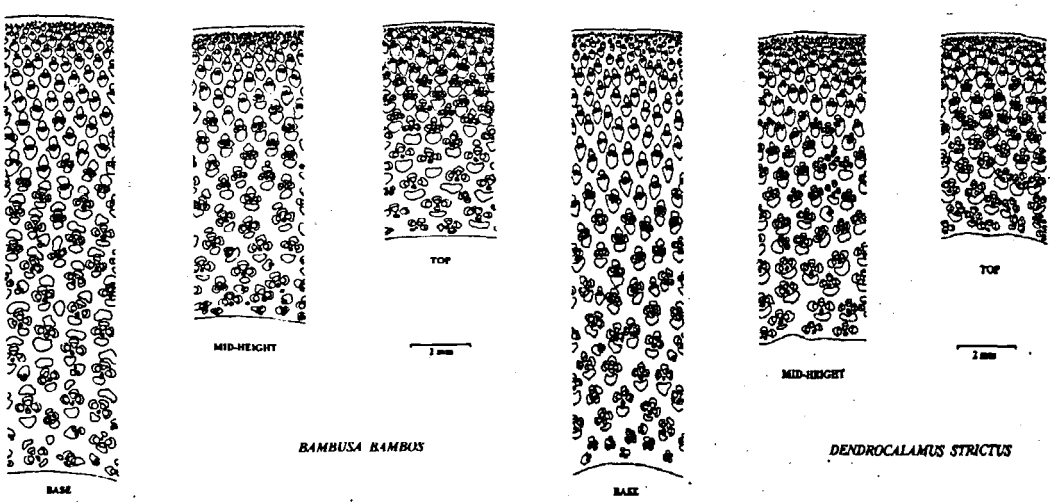

Fig. 1. Distribution of vascular bundles in different parts of the culm wall in *B. bambos* and *D. strictus*

were small and in *D. strictus* a few fibre strands were also found intermingled with these small vascular bundles. In outer one third portion of the culm wall, all the fibrous sheaths

of individual bundles were fused into ellipsoidal shaped strand structure. The vascular bundles in the mid-part of the culm wall in *B. bambos* were typically of type IV with six fibrous sheaths (designated as double broken-waist type) whereas *D. strictus* had type III bundles having five fibrous sheaths (known as broken-waist type). The bundle size was the greatest in this region and the fibre sheaths were prominent In the inner part of the culm wall, there was a slight reduction in bundle size and an irregular orientation of vascular bundles.

Table 2. Variation in tissue proportion at different height levels of the culm in *B. bambos* and *D. strictus*

	Bambusa bambos			*Dendrocalamus strictus*		
	Culm base	Mid-height	Culm top	Culm base	Mid-height	Culm top
Fibrous tissue (%)	33.87 (9.69)	38.81 (9.76)	35.28 (7.78)	29.18 (5.81)	32.85 (5.38)	39.31 (8.73)
Vascular tissue (%)	5.10 (1.92)	7.09 (1.64)	8.19 (1.88)	4.91 (1.50)	7.08 (1.31)	9.44 (1.67)
Ground tissue (%)	61.03 (10.4)	54.10 (9.02)	56.54 (7.78)	65.92 (5.86)	60.07 (5.53)	51.25 (8.04)
No. of bundles per cm^2	159.7 (58.9)	166.7 (64.7)	184.0 (53.12)	205.7 (42.3)	225.3 (61.08)	298.0 (57.50)

- Figures in parentheses show SD of the mean

Appreciable variation in bundle distribution and tissue composition was also found between different height levels of culms in both *B. bambos* and *D. strictus*. Notable among them was the increase in the number of bundles per unit cross sectional area and proportion of vascular tissue (Table 2) from culm base to top which resulted from more compact arrangement and reduction of average size of bundles. From several earlier studies also it has been found that the form, size and pattern of bundles are distinctly variable along the culm length and these changes are found to coincide with changes in culm wall thickness that occurs from culm base to the top (Liese, 1998).

As regards the fibre wall thickness and lignification which are indicative of fibre maturation, a similar gradation was evident from periphery towards interior parts of the

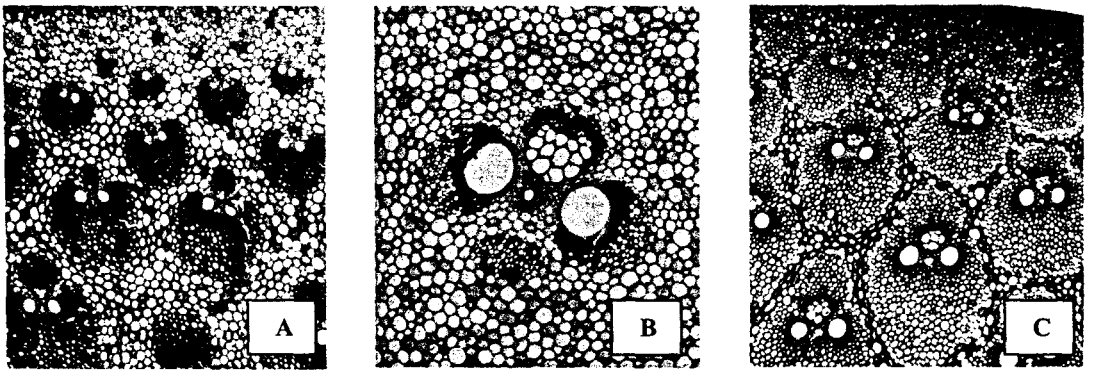

Fig. 2. Cross sections of 2-month-old culm of *D. strictus* showing vascular bundles with their fibrous sheaths: **A.** Culm base, outer part; note thick walled, lignified fibres x55. **B.** Culm base inner part with only few lignified fibres x55. **C.** Culm top, outer part with thin walled unlignified fibres x50.

wall at each height level at any particular culm age. Figs.2A and 2B reveal such differences in both morphology and maturity status of fibres in *D. strictus*. At the peripheral part of the culm wall, the fibres in the bundle sheath were more thick-walled and lignified, whereas in the inner parts, thickening of cell walls and lignification were limited to fibres in the immediate vicinity of vascular tissues. This difference probably has a partial role in imparting higher density and hardness to the outer shell of the culm wall as compared to the inner part at any stage of maturation. Further, it is also indicative of the sequential progress of cell wall maturation from peripheral part of the culm wall towards the inner side as observed in several other bamboo species (Alvin and Murphy, 1988; Majima *et al.*, 1991; Liese and Weiner, 1996, 1997; Murphy and Alvin, 1997a, b).

Besides the variation evident across the culm wall, remarkable difference was observed in fibre wall thickening and lignification between different height levels in both *B. bambos* and *D. strictus*. Comparison of figs.2A and 2C reveals the difference in cell wall thickening and lignification of bundle fibres in the outer part of the culm wall at basal (fig.2A) and top (fig.2C) portions of a two-month-old culm of *D. strictus*. While the fibrous sheaths of the bundles at the culm base at this stage consisted of thick-walled and lignified cells, those at the culm top comprised of relatively thin-walled and unlignified fibres. This observation supports the earlier finding (Alvin and Murphy, 1988; Itoh, 1990; Liese, 1998; Liese and Weiner, 1996) that cell wall thickening and lignification of fibrous tissues in the bamboo culm starts from its base and progresses upwards. Within an internode, however, these processes progress downwards (Itoh, 1990; Liese, 1998).

Structural changes during culm maturation

Mean fibre length between different age groups of *B. bambos* varied between 2897 μm and 3199 μm and that in *D. strictus* between 2909 μm and 3090 μm respectively and there was no definite trend of increase in fibre length in relation to increase in age of the culms from second month onwards. This suggests that whatever fibre elongation occurring during culm development is completed before the culms attain 2 months of age and there is no continued elongation of fibres subsequently. The elongation of fibres in bamboos is said to complete within a few days early during the differentiation of culm internodes (Hsiung *et al.*, 1980).

The most noticeable change in culm structure with increasing culm age was the thickening of cell walls of bundle sheath fibres and ground parenchyma tissues and their lignification. The fibres of the peripheral bundles of the culm wall were the first to undergo these changes. Subsequently, wall thickening and lignification of fibres progressed inwards in the radial direction and upwards in axial direction. Within a bundle, all the fibres did not mature simultaneously; in outer bundles of the culm wall where the vascular and fibrous tissues were fused into a composite structure, fibres lying adjacent to vascular tissue matured first and those away from the vascular tissues matured later (Fig.3A). In inner bundles, fibres flanking the protoxylem, metaxylem vessels and phloem underwent cell wall thickening and lignification first (Fig.3B). Thickening and lignification of fibre walls subsequently progressed to outer parts of the strand. In peripheral strand(s) of inner bundles, the fibres matured in a different sequence; those situated at the outer boundary of these strands were mostly the first to undergo cell wall thickening and lignification and the fibres in the core part followed a random pattern (Fig. 3B, at arrows).

The sequence of changes in cell wall structure during maturation of fibrous and ground tissues has been studied in great detail by Alvin and Murphy (1988), Majima *et al.* (1991), Liese and Weiner (1996,1997) and Murphy and Alvin (1997a, b). Based on these studies it is found that the maturation process proceeds quite differently over the cross section of the culm wall being influenced by the position of vascular bundles and the position of fibres within the bundle. Wall thickening of bundle sheath fibres within a bundle starts from inner vascular side and proceeds outwards.

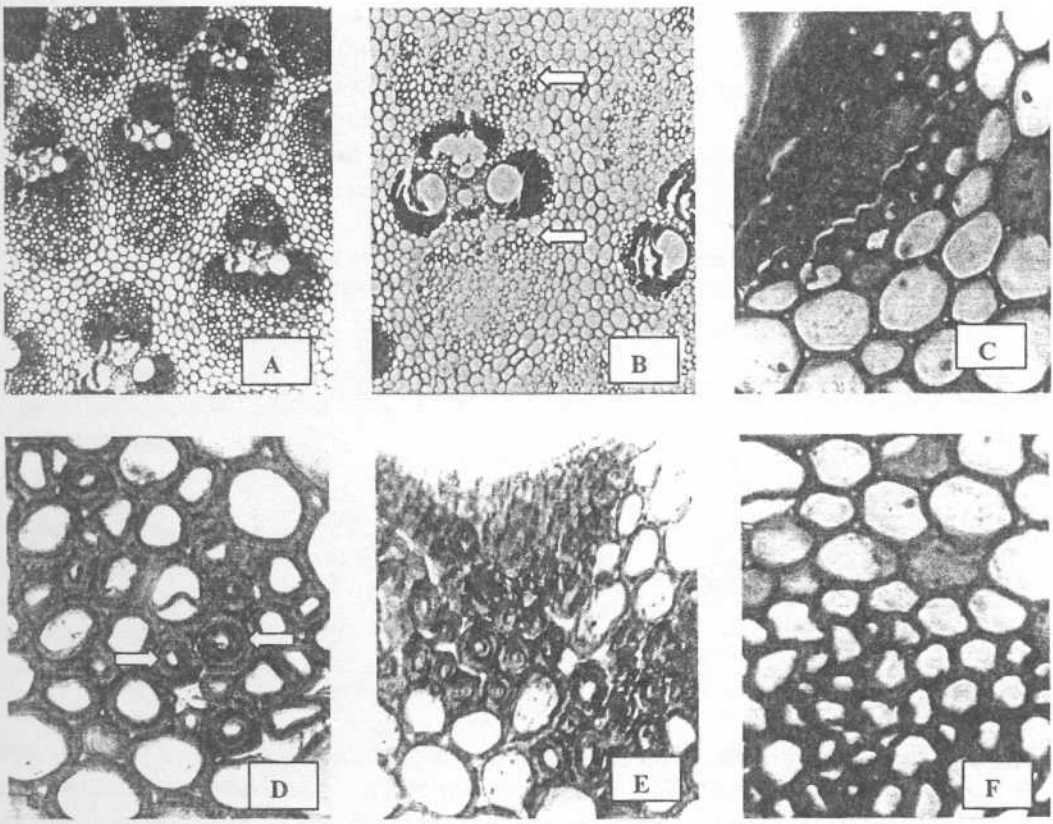

Fig. 3. A. Cell wall thickening and lignification in fibres in outer bundles in *B. bambos* x45. **B.** Initiation of wall thickening in fibres of peripheral fibrous strand in *B. bambos;* note few thick walled fibres at arrows x50. **C.** Fibres with polylamellate wall structure and thick-walled parenchyma cells in outer part of the 1-year-old culm wall in *D. strictus* x280. **D.** Polylamellate structure of thickened fibre walls(at arrows) in 5-year-old culm of *B. bambos;* note thick-walled ground parenchyma. x245. **E.** Fibre walls commonly with polylamellate cell walls and thick walled ground parenchyma in a 5-year-old culm of *D. strictus* x260. **F.** Relatively thin-walled fibres in fibro-vascular strands of inner part of a 5-year-old culm wall x280.

Thickening of fibre walls was accomplished by the deposition of additional cell wall lamellae as evident from the structure of fibre walls; some of the fibres within the outer strands showed polylamellate wall structure even in one-year-old material. Even the ground parenchyma cells showed thick cell walls (Fig.3C). In five-year-old culms highly thickened walls showing a distinct polylamellate structure and a narrow lumen were common in a large proportion of fibres in outer bundles (Fig. 3D, at arrows; Fig. 3E). Cell walls of the ground parenchyma were also thickened and lignified at this stage (Figs. 3D, 3E). However, even at the age of five years, the fibre walls of inner vascular bundles were

not fully thick-walled and lignified (Fig. 3F). It has been found that from several earlier studies (Murphy and Alvin, 1992, 1997a; Liese and Weiner,1996) that the fibre maturation is a process prolonged over many growing seasons and the number of lamellae in the cell walls continue to increase with culm age during, and even after culm maturation.

The thickening of fibre walls during culm maturation and ageing has been demonstrated by Liese and Weiner (1996, 1998)in *Phyllostachys viridioglaucescens* through measurement of fibre wall thickness in culms of different age groups. Since dimensions of fibres across the culm wall are highly variable at any stage depending on their position, careful characterization of fibre types was found important for measurement of their wall thickness. Generally, fibres contiguous parenchyma show polylamellate thick walls, those in contact with vascular tissue are narrow while those lying in the center are larger (Liese and Weiner, 1996). In the present study, the extent of cell wall thickening of three categories of fibres and ground parenchyma cells at culm base in different age groups of *B. bambos* and *D. strictus* was compared (Table 3).

Table 3. Changes in average double wall thickness (μm) of different types of fibres and ground parenchyma cells in culms of different age groups in *B. bambos* and *D. strictus*

Cell types	Bambusa bambos			Dendrocalamus strictus		
	2-month-old	1-year-old	5-year-old	2-month-old	1-year-old	5-year-old
Thick-walled fibres	8.43 (0.72)	9.13 (0.60)	9.30 (0.58)	7.95 (0.61)	7.39 (0.56)	7.82 (0.47)
Medium thin-walled fibres	7.65 (0.48)	8.13 (0.56)	9.08 (0.57)	6.87 (0.46)	7.94 (0.73)	7.97 (0.51)
Thin-walled fibres	5.63 (0.28)	6.02 (0.79)	6..93 (0.63)	3.84 (0.61)	5.05 (0.53)	7.41 (0.74)
Ground parenchyma cells	4.05 (0.56)	4.87 (0.76)	6.30 (0.39)	3.25 (0.75)	4.32 (0.69)	7.75 (0.31)

* Figures in parentheses show SD of the mean

From the table it is evident that, in general, there was an increase in cell wall thickness of culm tissues as age increased. While the increase in wall thickness of thick-walled fibres was either inconsistent or less pronounced, that of other categories of fibres and ground parenchyma cells showed a consistent increase from 2 months to 5 years of age. This suggests that cell wall thickening proceeds at different rates and at different age stages in various categories of fibres and parenchyma cells; thick-walled fibres are probably the first to undergo this process as supported by visual observation also. In ground parenchyma cells, wall thickening steadily increased between 2-month and 5-year age groups.

Age-related changes in physical properties

It is generally accepted that the changes in cell wall thickening and lignification of tissues accompanying increase in age of the culms have got direct influence on physical and mechanical properties of the culm material. It is found that the density increases and the moisture content decreases with increase in culm age (Liese, 1987b, 1998; Liese and Weiner, 1996). In order to know this relationship in *B. bambos* and *D. strictus,* the

average density and moisture content percentage values of different age groups were compared. The results are shown in Fig. 4.

It is clear from Fig.4 that the basic density was less than 300 kg/m^3 in 2-month-old culm material in both *B. bambos* and *D. strictus* which increased sharply to nearly 600 kg/m^3 during the initial two years. The subsequent increase in density was comparatively less pronounced in both the species. The moisture content percentage which showed an inverse relationship with density showed a sharp decline from a high value during the initial two years of culm age and subsequently maintained more or less a stable value. Moisture content percentage in 2-month-old *D. strictus* was as high as 475% whereas, it was about

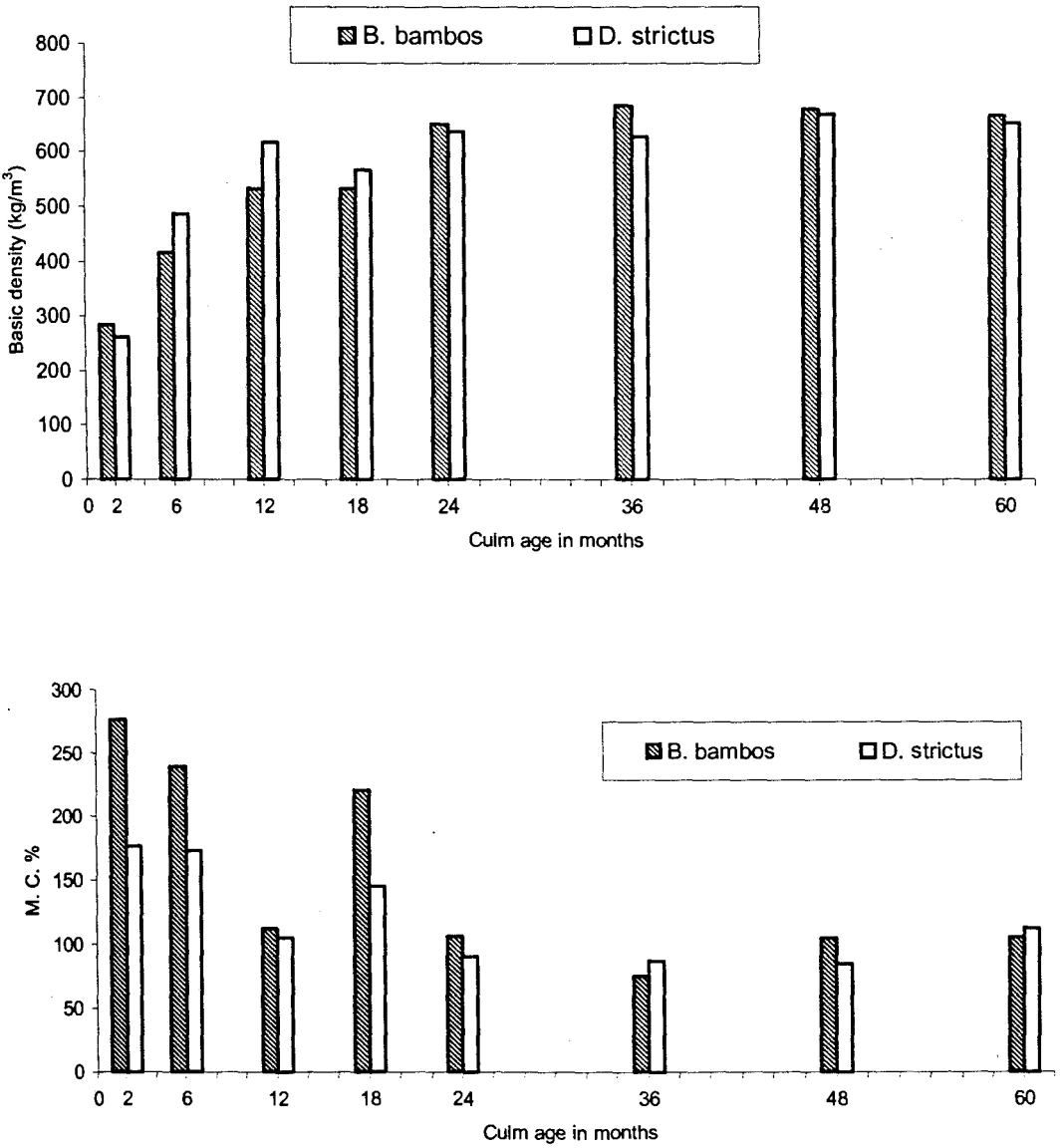

Fig. 4. Changes in density and M. C. % in relation to age in *B. bambos* and *D. strictus*

300% in *B. bambos*. Thus, the density and M.C.% curves in both the species indicate an initial phase of abrupt change up to the first 2 years which can perhaps be attributed to the maturation process of culm tissues. Microscopic examination of cell walls of fibres and ground parenchyma also support this observation since considerable cell wall thickening and lignification occurred during the first year of the culm development. Studies have shown that the developmental processes of culm maturation of bamboos are completed within two to three years and the cell wall thickening and lignification of tissues that occur subsequently in a second phase are related to the ageing process (Liese, 1987b, 1998; Liese and Weiner, 1996).

Storage metabolites in culm tissues

The main storage product in culm tissues in both *B. bambos* and *D. strictus* was starch which occurred as granules (Figs. 5A, 5B). In ground parenchyma tissue of the culms two types of cells namely, short cells and long cells could be distinguished. Starch was found only in long cells as found earlier by Liese (1998). Among the 21 culms of *B. bambos* collected, one culm each belonging to age groups of 6-months (Fig.5A), 1-year and 3-year age groups contained high starch. Moderate starch content was also found in 2-year and 5-year-old culms. In *D. strictus,* out of the 30 culms, highest starch content was observed in a 1-year-old culm (Fig.5B) whereas culms with moderate starch belonged mostly to 2-year and 3-year age groups. Chi-square test showed that status of starch was independent of culm age in both *B. bambos* ($\chi^2 = 20.90562$, $\chi^2_{21} = 33.9$) and *D. strictus* ($\chi^2 = 11.74$, $\chi^2_{14} = 23.68$). Thus, contrary to the finding that the starch content in culm tissues increases with culm age (Liese, 1998; Weiner and Liese, 1996), there was no definite relationship between occurrence of starch and age or maturity of culms in both *B. bambos* and *D. strictus*. With regard to the generally believed influence of period of collection (i.e., in

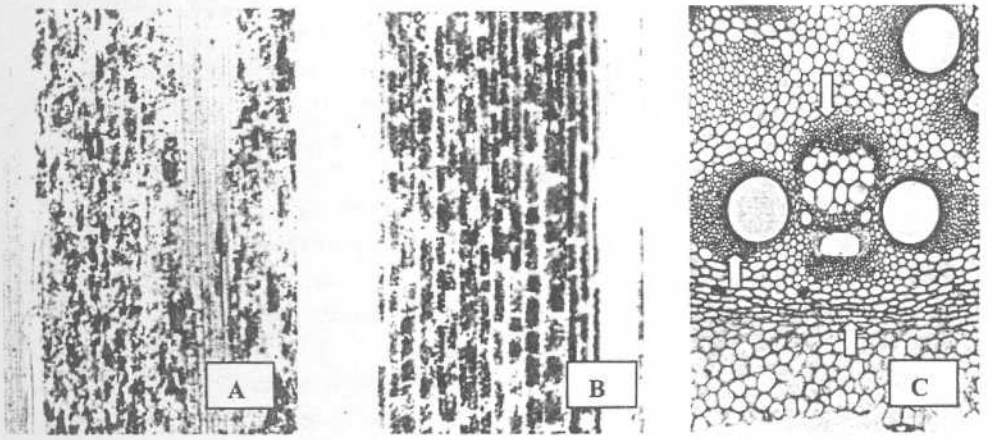

Fig. 5. A. Starch granules in ground parenchyma cells (LS) in a 6-month-old culm of *B. bambos* .x55. **B.** LS of a 1-year-old culm of *D. strictus* with abundant starch filling the cell lumens .x55. **C.** High protein content in fibres surrounding vascular elements and in the cells of the pith ring (at arrows). .x50.

terms of lunar fortnights) on starch content it was found that the two attributes were independent in *B. bambos* ($\chi^2 = 3.07$, $\chi^2_3 = 7.81$) and dependent in *D.strictus* ($\chi^2 = 6.25$, $\chi^2_2 = 5.99$). However, no definite conclusion could be drawn from the present observations on this inter-relationship.

None of the culm materials stored under laboratory condition showed borer infestation. Hence, correlation of the same with the starch content of the culm tissues could not be ascertained in the present study.

As evident from Sudan dye staining, lipids were confined to the cell membranes and no storage lipids were traceable as globules in cell lumens in any stage of culm development. On the other hand, total proteins were abundant in early stages of culm development particularly, in peripheral cortical layers, phloem and in fibres undergoing cell wall maturation. Cells of the pith ring were also found to contain abundant proteins in early stages of culm maturation (Fig.5C, at arrows). At subsequent stages, proteins were traceable only in the phloem tissue.

Morphological changes in culms with increasing age

Changes in culm morphological features with increasing age observed in the present study were helpful in deciding only the approximate age of culms; accurate age determination was not possible from these features owing to their gradual manifestation, local variations between sites and clumps and also clump age differences. Therefore, mere morphological features cannot be totally relied upon for culm age determination. However, since new culms are generally produced during a definite period of the year (usually during July-August in Kerala, after the first phase of monsoon) application of this knowledge can be helpful to reasonably increase the precision in culm age determination. The following are some generalized observations on external characteristics and other features helpful in culm age determination in *B. bambos* and *D. strictus*:

- In both *B. bambos* and *D. strictus* young culms up to about two to three months of age possess a white pubescent surface and intact culm sheaths covering the internodes; during subsequent part of the first year, culm sheath is shed and culm surface appears smooth, bright green. Culms of the current year are generally found along the perimeter of a clump.
- After the first year of growth the culm sheath is usually absent. Culm colour turns deep green and surface loses its smooth appearance and turns rather dull. In a clump, these culms occur in the sub-peripheral circle.
- A culm that is over two years of age has a dark green colour. Surface appears dull and hard. Such culms are usually located inner to the circle of one-year-old culms in a clump.
- Culms over three or more years of age usually have a dull green colour and a seemingly drier and hard surface appearance. Generally, they occupy the interior position within a clump.

CONCLUSIONS

From the anatomical observations and related data gathered in the present study it is concluded that:

1. Culm maturation in *Bambusa bambos* and *Dendrocalamus strictus* involves developmental changes such as cell wall thickening and lignification of culm

tissues; these changes do not occur simultaneously throughout the culm but follow a definite sequence across the culm wall and along the culm axis.

2. In both *B. bambos* and *D. strictus* maturation changes are rapid for the first two years before they slow down or stabilize from third year. This suggests that for harvesting mature bamboo, the culms should have completed at least two year's development.
3. Culm age is not the only factor influencing the status of storage starch in culm tissues.
4. External morphological features of culms can be used only in approximate age determination.

Acknowledgements

I acknowledge with deep gratitude the financial support from the Kerala Forest Department (Dev.), Thiruvananthapuram, to undertake this study. My sincere thanks are due to Dr. J. K. Sharma, Director, KFRI for his constant encouragement. I am also indebted to all my colleagues for their help and useful suggestions. The statistical advice and help in data analysis offered by Mrs. P. Rugmini. Division of Statistics, KFRI is sincerely acknowledged. The able technical assistance of Mr. K. K. Suresh and Mrs. P. K. Sujakumari who worked under the project during different periods is gratefully acknowledged. Thanks are due to Drs. K. M. Bhat, M. S. Muktesh Kumar and K. K. Seethalakshmi for their valuable editorial comments. I am also grateful to Dr. R. Gnanaharan, Research Coordinator, for his valuable suggestions.

REFERENCES

Abd. Latif, M. and Mohd. Zin, J. (1992). Culm characteristics of *Bambusa blumeana* and *Gigantochloa scortechinii* and its effects on physical and mechanical properties. *in* S. Zhu, W. Li, X. Zhang and Z. Wang (eds.) Bamboo and Its Use: Proc. Internat. Symp. on Industrial Use of Bamboo, Beijing, China, 7- 11 Dec. 1992. ITTO, and Chinese Acad. For., Beijing, China. pp. 118- 128.

Abd. Latif, M., Wan, T., Wan, A. and Fauzidah, A. (1990). Anatomical features and mechanical properties of three Malaysian bamboos. J. Trop. For. Sci. 2: 227- 234.

Agrawal, S. P. and Luxmi Chauhan (1990). Culm and leaf epidermis of Indian bamboos, Part III. *Melocanna baccifera* (Roxb.) Kurz. Indian For. 116: 815-818.

Alvin, K. L. and Murphy, R. J. (1988). Variation in fibre and parenchyma wall thickness in culms of the bamboo *Sinobambusa tootsik.* IAWA Bull. 9: 353-361.

Berlyn, G. P. and Miksche, J. P. (1976). Botanical Microtechnique and Cytochemistry. Iowa State Univ. Press, Iowa.

Espiloy, Z. (1987). Physico- mechanical and anatomical relationships of some Philippine bamboos. *in* A. N. Rao, G. Dhanarajan, and C. B. Sastry (eds.) Recent Research on Bamboo: Proc. Internat. Bamboo Workshop, Hangzhou, China, 6-14 Oct. 1985. Chinese Acad. For. , Beijing, China, and IDRC, Ottawa, Canada. pp.257-264.

Espiloy, Z. (1992). Properties affecting bamboo utilization. in S. Zhu, W. Li, X. Zhang and Z. Wang(eds.) Bamboo and Its Use: Proc. Internat. Symp. on Industrial Use of Bamboo , Beijing, China, 7-11 Dec. 1992. ITTO, and Chinese Acad. For., Beijing, China. pp. 139- 142.

Espiloy, Z. (1994). Effect of age on the physico- mechanical properties of some Philippine bamboo. in Bamboo in Asia and Pacific: Proc. 4th Internat. Bamboo Workshop, Chiangmai, Thailand, 27-30 Nov. 1991. IDRC, Ottawa, Canada and FORSPA, Bangkok, Thailand. pp.180- 182.

Fujii, T. (1985). Cell wall structure of the culm of Azumanezasa (*Pleioblastus chino* Max.). Mokuzai Gakkaishi 31: 865-872.

Ghosh, S. S. and Negi, B. S. (1959). Anatomical features of bamboo used for paper manufacture. Cell. Res. Symp. CSIR, New Delhi, India, pp. 139- 148.

Ghosh, S. S. and Negi, B. S. (1960). Anatomy of Indian bamboos, Part I. Epidermal features of *Bambusa arundinacea* Willd., *B. polymorpha* Munro, *B. vulgaris* Schrad., *Dendrocalamus membranaceous* Munro, *D. strictus* Nees and *Melocanna bambusoides* Trin. Indian For. 86: 719-727.

Ghosh, S. S. and Negi, B. S. (1961). Occurrence, distribution and other characteristics of septate fibers in monocotyledons with special reference to *Dendrocalamus strictus* Nees. Proc. 48th Industrial Science Conference, Part III: pp.277-278

Gomori, G. (1952). Microscopic Histochemistry: Principles and Praactice. Univ. Chicago Press, Chicago.

Grosser, D. and Liese, W. (1971). On the anatomy of Asian bamboos with special reference to their vascular bundles. Wood Sci. Technol. 5: 290- 312.

Grosser, D. and Liese, W. (1973). Present status and problems of bamboo classification. J. Arnold Arboret. 54: 293- 308.

Grosser, D. and Zamuco, G. I. (1973). Anatomy of some bamboo species in the Philippines. Philippine J. Sc. 100: 57-72.

Hsiung, W. Y., Quiao, S. Y. and Li, Y. F. (1980). The anatomical structure of culms of *Phyllostachys pubescence* Mazel ex H. de Lehaie. Acta Botanica Sinica 22: 343-348.

Itoh, T. (1990). Lignification of bamboo (*Phyllostachys heterocycla* Mitf.) during its growth. Holzforschung 44:191- 200.

Janssen, J. J. A. (1981). The relationship between the mechanical properties and the biological and chemical composition of bamboo. *in* T. Higuchi (ed.) Bamboo Production and Utilization: Proc. XVII IUFRO World Congress Kyoto, Japan, 6- 17 Sept. 1981. Kyoto Univ., Kyoto, Japan. pp. 27-32.

Jiang, X. (1992). The Microstructure of Main Chinese Bamboos. Chinese Acad. For. Beijing, China

Jiang, X. and Li, Q. (1983). Preliminary observations on vascular bundles of bamboos native to China. J. Sichuan Agri. College, 1:57-68.

Johansen, D. A. (1940). Plant Microtechnique. McGraw Hill, New York.

Lee, C. L. and Chin, T. C. (1960). Anatomical studies on some Chinese bamboos. Acta Botanica Sinica 9: 76-95.

Liese, W. (1980). Anatomy of Bamboo. *in* G. Lessard and A. Chouinard (eds.) Bamboo Research in Asia. Proc. Workshop held at Singapore, 28-30 May 1980. IDRC, Ottawa, Canada. pp.165-172.

Liese, W. (1987a). Research on bamboo. Wood Sci. Technol. 21:189-209.

Liese, W. (1987b). Anatomy and properties of bamboo. *in* A. N. Rao, G. Dhanarajan and C. B. Sastry (eds.) Recent Research on Bamboos: Proc. Internat. Bamboo Workshop, Hangzhou, China 6- 14 Oct.1985. Chinese Acad. For. Beijing, China and IDRC, Ottawa, Canada pp. 196-208.

Liese, W. (1997). The protection of bamboo against deterioration. *in* P. K. Jena (ed.) Proc. 2nd Internat. Conf. On Non-conventional Building Materials (NOCMAT-97)Bhubaneswar, India, 17-19 June, 1997 Natural Resources Development Foundation, Bhubaneswar, India, pp.111-117.

Liese, W. (1998). The Anatomy of Bamboo Culms. Technical Report, INBAR, Beijing, China.

Liese, W. and Weiner, G. (1996). Ageing of bamboo culms. A review. Wood Sci. Technol. 30: 77-89.

Liese, W. and Weiner, G. (1997). Modification of bamboo culm structures due to ageing and wounding. in G. Chapman. (ed.) The Bamboos. Linnaean Society. London, UK. pp. 313- 322.

Majima, S., Fugita, M. and Saiki, H. (1991). The cell wall maturation in moso bamboo (*Phyllostachys pubescence* Mazel) and the occurrence of peroxidase related to lignification. Bull. Kyoto Univ., Kyoto Univ. Press, 63: 236- 244.

Metcalfe, C. R. (1960). Anatomy of the Monocotyledons. I. Gramineae. Oxford University Press, USA

Muller, L. (1996). Vascular bundle arrangement, an aid to bamboo recognition. American Bamboo Society Newsletter, 17: 6-9.

Murphy, R. J. and Alvin, K. L. (1992). Variation in fibre wall structure in bamboo. IAWA Bull. n.s. 13: 403- 410.

Murphy, R. J. and Alvin, K. L. (1997a). Fibre maturation in the bamboo *Gigantochloa scortechinii*. IAWA J. 18: 147- 156.

Murphy, R. J. and Alvin, K. L. (1997b). Fibre maturation in bamboos. *in* G. Chapman (ed.) The Bamboos. Linnaean Society, London, UK. pp. 293- 303.

Ota, M. and Sugi, S. (1953). On the arrangement of the vascular bundle in the bamboo stem. Report of Kyushi Univ. For. 1:79-86.

Parameswaran N. and Liese, W. (1975). On the polylamellate structure of parenchyma wall in *Phyllostachys edulis*. IAWA Bull. 4: 57- 58.

Parameswaran, N. and Liese, W. (1976). On the fine structure of bamboo fibres. Wood Sci. Technol. 10: 231-246.

Parameswaran, N. and Liese, W. (1977). Structure of septate fibres in bamboo. Holzforschung 31: 55- 57

Parameswaran, N. and Liese, W.(1980). Ultrastructural aspects of bamboo cells. Cellulose Chem. Techn. 14: 587-609

Pattanath, P. G. (1972).Trend of variation in the fibre length in bamboos. Indian For. 98: 241- 243

Pattanath, P. G. and Ramesh Rao, K. (1969). Epidermal and internodal structure of the culm as an aid to identification and classification of bamboos. *in* Recent Advances in the Anatomy of Tropical Seed Plants. Hindustan Publishing Corporation (India) Delhi. pp. 79-196.

Preston, R. D. and Singh, K. (1950). The fine structure of bamboo fibers. I. Optical properties and x-ray data. J. Exp. Bot. 1:214-226.

Qiao, S. (1991). Observation on vascular bundles of 52 bamboo species. Bamboo Res. 10: 84- 100.

Rong, P. R. (1985).Comparative observation on the anatomical structure of culms of *Phyllostachys pubescence* and of its several varieties. J. Bamboo Res. 4: 89- 97.

Sattar, M. A., Kabir, M. F. and Battacharjee, D. K. (1994). Effect of age and height position of muli (*Melocanna baccifera*) and borak (*Bambusa balcooa*) bamboos on their physical and mechanical properties. *in* Bamboo in Asia and Pacific: Proc. 4th Internat. Bamboo Workshop, Chiangmai, Thailand, 27- 30 Nov. 1991. IDRC, Ottawa, Canada and FORSPA, Bangkok, Thailand. pp.183- 187.

Sekar, T. and Balasubramanian, A.(1994a). Culm anatomy of *Guadua* and its systematic position. BIC-India Bull. 4: 6-9.

Sekar, T. and Balasubramanian, A.(1994b).Culm anatomy of *Melocanna baccifera* (Roxb.) Kurz. BIC-Indla Bull. 4: 10- 13.

Sekar, T. and Balasubramanian, A.(1994c). Structural diversity of culm in *Bambusa vulgaris.* J. Indian Acad .Wood Sci. 25: 25- 31.

Shigematsu, Y.(1940). On the length and width of the fibre of kanchiku (*Chimonobambusa marmorea*). J. Japan For. Soc. 22:693-696

Sulthoni, A. (1987). Traditional preservation of bamboo in Java, Indonesia. *in* A. N. Rao, G. Dhanarajan and C. B. Sastry (eds.) Recent Research on Bamboos: Proc. Internat. Bamboo Workshop, Hangzhou, China 6- 14 Oct. 1985. Chinese Acad. For. Beijing, China , and IDRC, Ottawa, Canada. pp. 349- 357.

Tamolang, F. N., Valbuena, A. B., Lomibao, A. B., Artuz, A. E., Kalaw, C. and Tongacan, A. (1958). Fibre dimensions of certain Philippine broadleaved and coniferous woods, palms and bamboos II. Tappi 41:614-621.

Tono, T. and Ono, K.(1962). The layered structure and its morphological transformation by acid treatment. J. Japanese Wood Res. Soc. 8: 245-249.

Velasquez, G, and Santos, S. (1931). Anatomical study of the culm of five Philippine bamboos. Natural and Applied Science Bull. Univ. Philippines 1(4) :281-296.

Wang, Y. C., Hsieh, J. S. and Wu, S. C. (1994). Structural variability of vascular bundles of some exotic bamboo species. *in* Bamboo in Asia and Pacific. Proc. 4th International Bamboo Workshop, Chiangmai, Thailand, 27-30 November 1991. IDRC, Ottawa Canada and FORSPA, Bangkok, Thailand. pp. 222-226.

Weiner, G. and Liese, W. (1996). Wound reactions in bamboo culms and rhizomes. J. Trop. For. Sci. 9: 379-397.

Wen Taihui and Chou Wenwei (1984). A report on the anatomy of the vascular bundle of bamboos from China.(1) . J. Bamboo Res. 3: 1- 21.

Widjaja, E. A. and Risyad, Z. (1987). Anatomical properties of some bamboos utilized in Indonesia. *in* A. N. Rao, G. Dhanarajan and C. B. Sastry (eds.) Recent Research on Bamboos: Proc. Internat. Bamboo Workshop, Hangzhou, China, 6- 14 Oct. 1985. Chinese Acad. For., Beijing, China, and IDRC, Ottawa Canada. pp.244 - 246.

Wu, S. C. and Hsieh, J. S. (1990). The structural variation of recently introduced bamboosin Taiwan. For. Prod. Industry 9: 55-78

Wu, S. C. and Leu, J. L. (1987). The ultrastructure of vascular bundles of some Taiwan bamboo species. Quart. J. Exp. For. NTU 1: 21-44.

Wu, S. C., Leu, J. L. and Hsieh, J. S. (1992). Relationships between anatomical characteristics and permeability properties in Taiwan grown bamboo species. *in* S. Zhu, W. Li, X. Zhang and Z. Wang (ed.) Bamboo and its Use. Proc. Internat. Symp. Industrial Use of Bamboo. Beijing, China 7-11 Dec.1992 ITTO, and Chinese Acad. For., Beijing, China. pp. 133-138.

Younus-uzzaman, M. (1991). Anatomical features of *Arundinaria falconeri* and *Sinobambusa tootsik* with reference to their effects on penetration of preservatives. J. For. Sci. 20: 39- 44.

Zhang, J., Wang, R., Ma, N. and Zhang, W. (1995). Fibre morphology and main physical and chemical properties of some bamboo wood of *Phyllostachys*. For. Res. 8: 54- 61.