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Anatomy of Cane Internodes of Tiger Grass [*Thysanolaena maxima* (Roxb) Kuntze]

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Abstract

In this study, we investigated the anatomical properties of cane internodes of *Thysanolaena maxima* (Roxb) Kuntze in respect of phloem, xylem, metaxylem, fiber band thickness; vascular bundle distribution, shape and size; and changes due to age of canes. Cane internodes consisted of parenchyma ground tissues, and scattered vascular bundles. The cane internodes consisted of parenchyma, fiber and conducting tissue in a ratio of 8.13: 3.37: 1.00, respectively. Parenchyma content decreases in the older cane internodes due to the deposition of secondary wall matters. Epidermal cells were elongated in anticlinal direction and closely attached to form a compact layer devoid of intercellular spaces. Hypodermal sclerenchyma was considerable and provided stiffness to the cane. There was a steady increase of thick wall cells from top to basal internodes of a cane. Vascular bundle consisted of xylem with two large metaxylem vessels and one to three protoxylem elements which with the age of the internodes obliterated to form lacuna; and the phloem with thin walled unlignified sieve tubes connected with companion cells. Radial length of vascular bundles was always greater than tangential. Density of vascular bundles was 1 in 10 mm^2 area of the outer 1/4th of a cut while it is 1 n 70 mm^2 in the inner 3/4th of a cut. Three different types of vascular bundles were noted in respect of bundle sheath distribution. Metaxylem vessel lumen gradually reduced with the age of cane internodes. Number and sizes of xylem tissue per vascular bundle was greater than phloem components. Phloem was highly organized; sieve tubes remain functional throughout the life time of a cane. These findings reflect that the tiger grass cane has efficient to keep culms erect and develop food reserve system.

INTRODUCTION

Thysanolaena maxima (Roxb) Kuntze belongs to family Poaceae (syn. Gramineae). Locally, it is known as 'Phuljharu' due to its common use as floor sweeper in Bangladesh. It is known as tiger grass/bouquet grass/broom grass in English (Tiwari *et al.*, 2012). It is also known as 'Kunchi' in the Indian central himalaya and 'pildi', 'naltura' and 'narkatia' in South/West India. The species occur in worm temperate and sub-tropical parts of India, Bhutan, Burma, China, East Asia, Nepal, New Guinea and Malaysia up to 2000 m from sea level. In Bangladesh, it is found in large number as natural vegetation in the Chittagong and Chittagong hill track districts. Due to its popular use as floor sweeps in Bangladesh, it is even cultivated in hilly region of Chittagong division. It is considered as an important grass species due to its many ecological and economic functions. Tiger grasses usually grow in wide range of habitats. It can grow in soils having 5.3 to 9.3 pH. It survives in wide range of moisture in the habitat. It can grow in 11.6 to 37.6% moisture range which is an unique in adaptation. It is successfully flourishes in 0.4 -2.7% carbon regime. As regards anatomy, there are no differences existing in leaf anatomy among naturally growing plants and those *in vitro* raised plants (Bhuchor, 2011). Leaf structure likes that of C_3 plants, strong bulliform system with absent of Kranz anatomical features are the main leaf anatomy. Maximum growth occurs in July to September and during this period translocation efficiency is high. Multiple leaf formation occurs during monsoon season but rhizome formation is profound during winter season.

The culm of grasses is typically hollow cylinders interrupt at interval by transverse partition. Transverse section show the culm is built up essentially of parenchymatous ground tissue. The scattered vascular bundles are collateral with phloem at the pole of the bundle towards the periphery of the culm, where it is usually embedded in fibrous tissue. Velican *et al*. (1966) discussed transverse diameter of parenchyma and sclerenchyma cells in relation to culm strength in rice.

T. maxima (Roxb) Kuntze reduces water runoff and soil loss from degraded land and hilly slopes. Conservation value as reported is 53.1% and 38.0% for water runoff and soil loss respectively (Bhuchor, 2011). There is no work available in cane internode anatomy. The present work was under taken to describe the transverse area of four top most cane internodes and to measure the area of phloem, xylem, metaxylem, fiber band thickness; vascular bundle number, size and shape in each cut.

MATERIALS AND METHODS

Plant samples were collected from naturally grown *T. maxima* (Roxb) Kuntze in the hilly districts of Bangladesh. Ten stands were randomly selected from three different cites of Chittagong hill tracts districts. From each stands one middle cane as collected having 14 internodes from the ground. Top four visible internodes (viz. Internodes 14, 15, 16 & 17 from the ground) were collected; 2 cm in length from the base of each cane internodes were taken for anatomical study.

The materials were fixed in formaldehyde acetic alcohol fixative (FAA, formaldehyde 10%, acetic acid 5%, ethanol 50% and distilled water 35%) for 48 hours and subsequently transferred to acetic alcohol solution (acetic acid 25% and ethanol 75%) for a long term storage. Free hand sections were prepared by a series of dehydration in ethanol using single-stained technique with Safranine stain. Measurements were taken with a Motic advanced biological research microscope through image analysis using *Motic J1.0* image processing software installed in Macintosh computer. Data on anatomical traits were recorded using all 10 stands in each sample area. The data of the traits were recorded on dermal tissue, vascular tissue, ground tissue and mechanical tissue.

RESULTS AND DISCUSSION

The properties of *T. maxima* (Roxb) Kuntze cane internodes are determined by its anatomical structure. The cane consists of both internodes and nodes. Twenty four to thirty internodes were recorded but it was varied with the situation in which they grow. Average numbers of internodes were 28.17 ± 1.49 (Figure1) at the time of flowering. The outer most part of the culm was formed by single layer of epidermal cells; the inner side is covered by 4-5 layers of parenchyma cells followed by 10-12 layers of sclerenchyma cells (Fig. 1). The gross anatomical structure of a transverse section of any internodes' was determined by the vascular bundles, their shape and sizes, arrangement, and numbers. The vascular bundles contrast the parenchymatous ground tissue, which is much lighter in colour. Extreme peripheral zone of the culm, the vascular bundles are small (Fig. 1). This agreement is similar to other reports in bamboo (Liese, 1980), wild sugarcane (Hossain 2010). In general, the vascular bundles were numerous in peripheral zones, larger, except extreme periphery; at the inner part, larger and fewer (Fig. 1). The total vascular bundles decrease from basal internodes to distal internodes within a culm. The average 139 ± 9, 131 ± 7, 124 ± 11 and 119 ± 14 vascular bundles were counted respectively from internode-14 to internode-17 (Fig. 1). It appears that the pattern of vascular bundles distribution in any internode was comparable to that of bamboo (Liese, 1980; Abd Latif *et al*., 1992). The internodal region of the cane was solid in nature and the vascular bundles in a cross section were scattered throughout the ground tissue instead of cylindrical arrangement. Scattered vascular bundles in the culm ground tissue have been reported in *Saccharum spontaneum* and *Pennisetum purpureum* (Hossain 2010).

The cane consisted of parenchyma cells forming the ground tissue and the vascular bundles composed of vessels, sieve tubes with companion cells and fibers. The total cane internodes consisted of 65% parenchyma, 27% fibers and 8% conducting cells viz. vessels, and sieve tubes. In general, the parenchyma content decreased and the amount of fiber increased from top internodes to bottom internodes (Fig. 2). Scalarification of ground tissue in older internodes in Napier grass and wild sugarcane are very prominent which lead to increase in lignified cells (Hossain, 2010). In bamboo, the amount of fiber increases from bottom to top, with the parenchyma content decreasing in the vertical direction (Liese, 1980; Abd. Latif and Mohd. Tamizi, 1992).

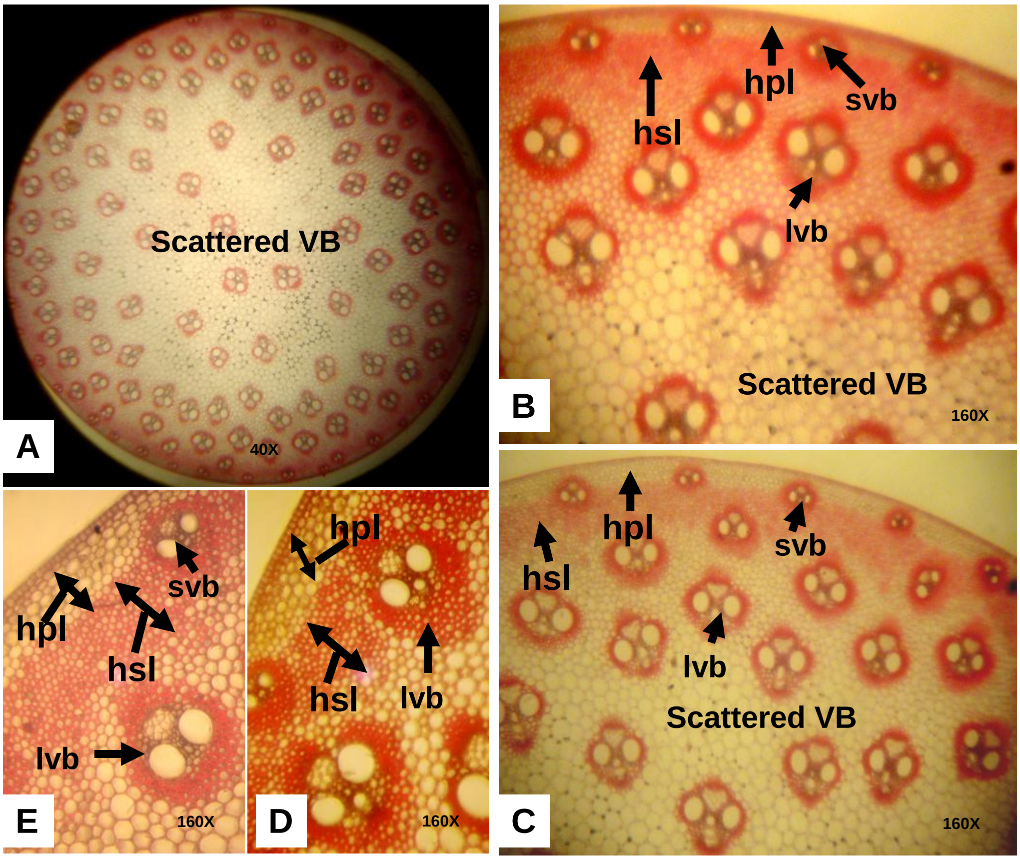


Figure 1. Transverse section of cane internode showing scattered vascular bundles in the ground tissue. A. Transverse section of internode-14. B. A part of internode-14 showing closer look of vascular bundles. C. Part of transverse section of internode-15. D. Part of transverse section of internode-16 and E. Part of transverse section of Internode-17. hpl = hypodermal parenchyma layers; hsl =hypodermal sclerenchyma layers; svb = small vascular lvb= large vascular bundles.

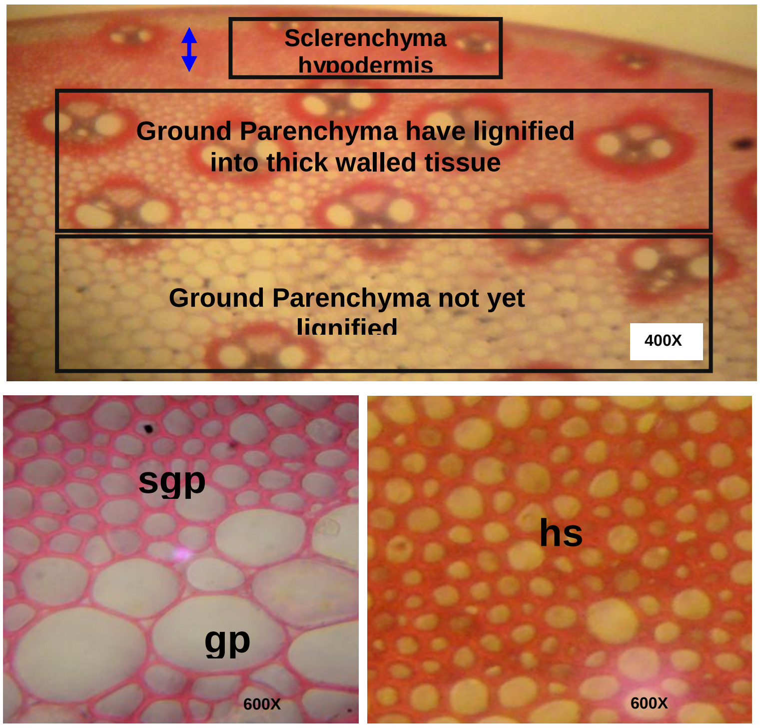


Figure 2. Showing lignification of ground parenchyma. sgp = sclerified ground parenchyma; gp = ground parenchyma; hs = hypodermal sclerenchyma.

Epidermal cells were elongated in anticlinal direction covered with heavy cuticle (Fig. 3). Outer anticlinal wall was thicker than inner anticlinal wall. There is a gradual increase in anticlinal length of epidermal cells from top internode towards the bottom internodes. The measurement of anticlinal length were 0.92 ± 0.15 mμ, 1.16 ± 0.12 mμ, 1.24 ± 0.27 mμ and 1.37 ± 0.31 mμ, respectively from internode-17 (top most) to internode-14 (Fig.2). Gradual sclerification of hypodermal parenchyma was noted from top internode to down internode-14 (Fig. 3). The epidermal cells were radially elongated in wild sugarcane and Napier grass (Hossain, 2010). The epidermal cells were closely attached to form a compact layer devoid of intercellular spaces in *T maxima*.

Hypodermal parenchyma consists of 6-7 layers of cells in the top internode but there were gradual increase in layer number and width towards the bottom internodes (Fig. 3). It was due to gradual scalarification in hypodermal parenchyma as the internodes become older and older. It is a xerophytic adaptation as noted in bamboo and grasses (Liese, 1980; Hossain, 2010). Hypodermal sclerenchyma was considerable in experimental plant. It ranged from 6-9 cell layers in distal internodes (Fig. 3). There was a steady increase of thick walled cells from top culm internodes to bottom internodes. Ground parenchyma became thickened due to deposition of secondary wall materials. Increase in thick walled ground tissue have been reported in bamboo (Liese, 1980), Napier grass (Hossain, 2010), rice (Joarder, 1980) and grasses and cereals (Metcalfe, 1960).

The ground tissue consisted of parenchyma cells which are mostly vertically elongated and possesses thicker wall which became lignified in the early stages of shoot growth (Fig. 3). Some short cube like parenchyma was noted towards the outer regions of the internodes (Fig. 1 and 3). Similar shorter cells were reported in bamboo characterized by a dense cytoplasm and thin cell wall, but they did not lignify even in mature culm and retain their cytoplasm for a long time (Liese, 1980). The ground parenchyma in the central region of the culm was compactly arranged with little or no intercellular spaces. In older stem, the cell wall became thickened due to secondary deposit. Parenchyma cells were connected with each other by small simple pits located on the longitudinal walls.

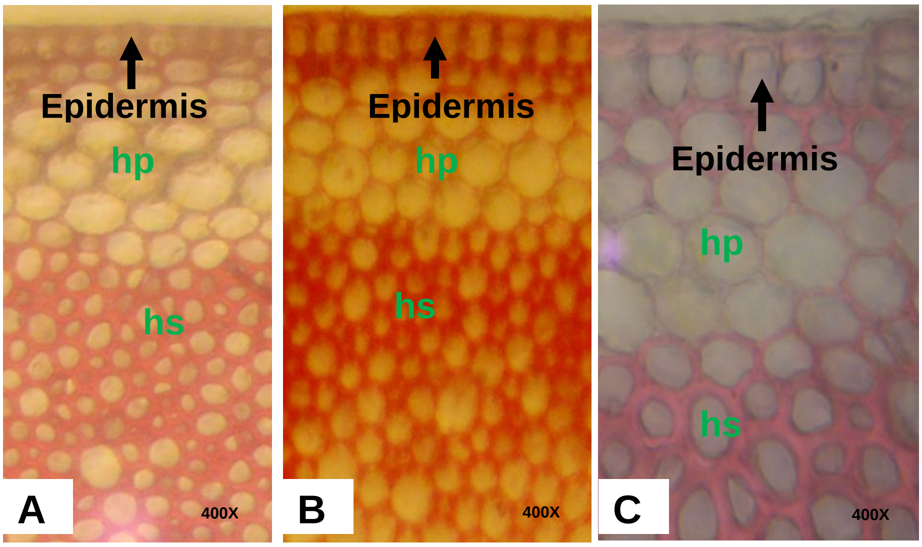
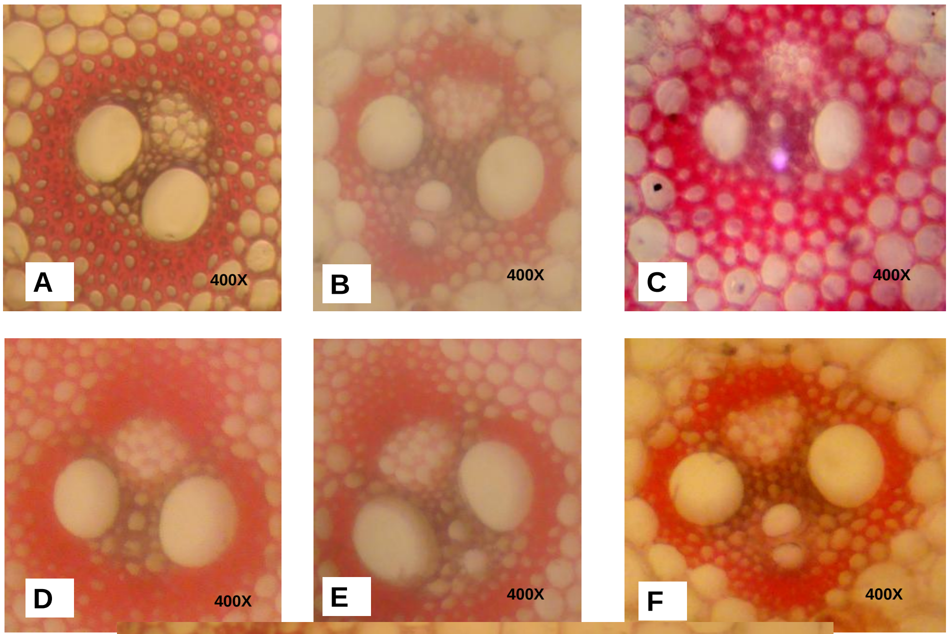


Figure 3. A, B and C: Part of transverse section showing epidermis and hypodermal regions of cane internodes; hp = hypodermal parenchyma, hs = hypodermal sclerenchyma

Vascular bundles in the culm internodes consisted of xylem with two large metaxylem vessels with one or two protoxylem elements and the phloem with thin walled, undignified sieve tubes connected with companion cells (Fig. 4 and 5). The vascular bundles in the parenchymatous hypodermis were the smallest and became larger towords the inner part. Area measured in hypodermis was 1754 ± 41.49 $mμ^2$ and in centre was 2433 ± 247 $mμ^2$ (Fig. 3 and Fig. 4). Hypodermal vascular bundles are smaller in rice, wheat, Napier, wild sugarcane and bamboo (Joarder, 1980; Joarder and Sima, 2011; Hossain, 2010; Liese, 1980; Grosser and Liese, 1971; Latif *et al*.., 1990, 1992). Sizes of vascular bundle did not change significantly with the ages of the culm. The radial and tangential ratio as measured and proportional values were in 1.13 : 1.00 and 1.48 : 1.00 in distal and basal internodes respectively but no significant differences in hypodermal small vascular bundles were noted (Fig. 3 and Fig. 4). The radial/tangential ratio of vascular bundles exhibited significant correlation with age and height of culm in bamboo (Latif *et al*. 1992; Latif and Tamiza, 1992).



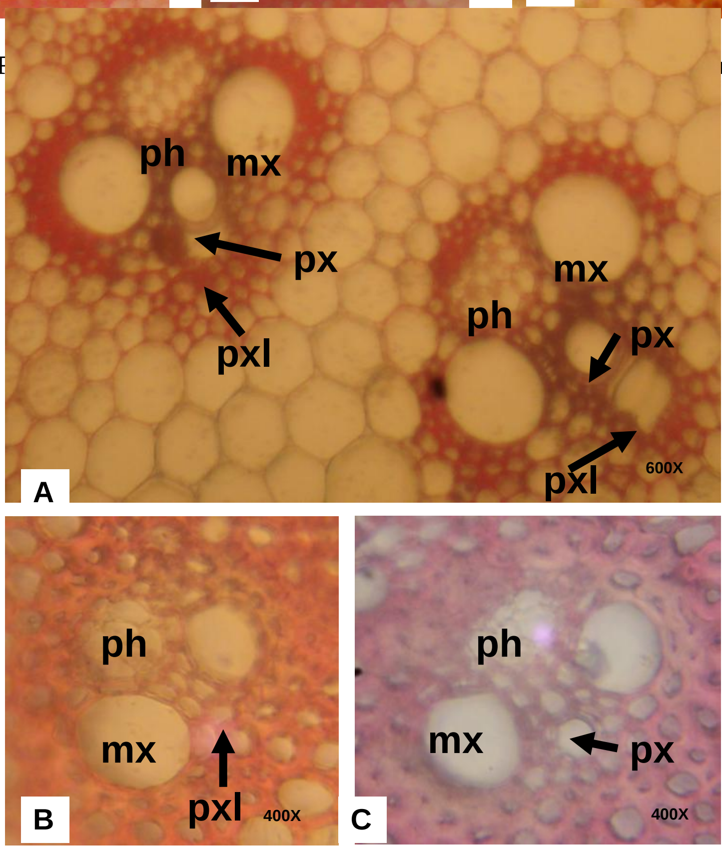


Figure 5. A. Large vascular bundles in the centre of ground tissue. B, C. Hypodermal small vascular bundles completely embedded in fibrous tissue; ph=phloem, px=protoxylem, mx=metaxylem, pxl=protoxylem lacunae.

The density of vascular bundle was 1 in 10 mm^2 areas of the outer 1/4th of the internode section and 1 in 70 mm^2 in the inner 3/4th of the cane internode section. No differences were noted in density of vascular bundles with the age of the culm internode. Grosser and Lies e (1971) noted higher density of the vascular bundles in the distal internodes of bamboo due to the decrease in culm wall thickness. In Napier grass and wild sugarcane, vascular bundles are denser in the peripheral regions of culm internodes (Hossain, 2010).

Both vessels and phloems were surrounded by sclerenchyma sheath (Fig. 4 and 5). They differ considerably in size, shape and position in the cane internode. Vascular bundles completely surrounded by sclerenchyma tissue were more frequent in the hypodermal region and small bundles were of this type. Bundle sheath and vascular tissue ratio was 1.78: 1.00. Vascular bundle consisted of central vascular stand with sclerenchyma sheath which is thicker in phloem; metaxylem and protoxylem regions were also observed (Fig. 4B and Fig. 5A). Frequency of occurrence of this type was 22.66%. The bundle sheath and the central vascular tissue ratio was 1.31: 1.00. This type was more frequent in older internodes. Liese (1980), and Grosser and Liese (1971, 1974) analyzed the variability of vascular bundles form and size ; and grouped them into four basic types in bamboo. They noted considerable variability in the appearance of the vascular bundles within one culm and we noted some similarities as regards vascular bundle forms and sizes in Tiger grass. In Tiger grass, vascular bundle variation was related to stages of development and maturity.

Vascular bundles have two large metaxylem and one or two intact protoxylem vessels. Intact protoxylem vessel was common in distal cane internodes but in older/basal internodes protoxylem gets dissolved to form lacunae (Fig. 4 and Fig. 5). Metaxylem vessel area larger at inner part of the cane internodes (area ranged from 321 – 349 $m\mu^2$) and became smaller towards the outer part of cane internodes (area ranged from 219 – 271 $m\mu^2$). Again, the vessel lumen was gradually smaller in the older canes due to increase in wall thickness. Liese (1980) and Grosser and Liese (1971, 1974) noted larger vessels in the inner and small in the outer part of bamboo culm internodes. The vessel wall thickness was 13.49 – 17.20 mμ in the largest and 12.77 – 16.44 mμ in the smallest vessels. Wall thickness was much more in the older cane internodes. The conducting tissue was functioning throughout the life time of a cane, without addition of any new conducting tissue, a feature very common in bamboo (Liese, 1980; Latif *et al.*, 1992). No blocking of conducting tissue in older internodes was noted as reported in bamboo (Liese, 1980).

The xylem and phloem tissue per vascular bundle was 38.14% and 14.59% respectively in smaller bundles whereas these were 43.71% and 15.35% for large bundles. Higher xylem conducting tissue indicated Tiger grass needs lot of water in order to survive as it needs multiple vascular bundles that are smaller in size in order to absorb excess water. It is an adaptation to survive during extreme summer situation. Smaller bundles are considered to be more efficient to conduct water to a great height in bamboo (Liese, 1980). Phloem was highly organized and had regular pattern of narrow companion cells and wide sieve tube cells. Sieve tubes remain functional throughout the life time of a cane internode without any blocking by tylosoid-like out growth which occurs frequently in older bamboo culms (Liese, 1980; 1987).

Fibre wall thickness was not significantly affected by either age or height but some decrease in lumen diameter with the increase in height and age was decreased. The average fibre wall thickness ranged from 3.5 – 6.5 mμ. Decrease of sclerenchyma cello lumen with the age and height have been reported in wild sugarcane (Hossain, 2010), cereals (Joarder, 1980, Joarder and Sima, 2011) and bamboo (Latif *et al*. 1980)

CONCLUSION

Tiger grass (*Thysanolaena maxima*) usually grows in hilly regions of Bangladesh but can grow well in plain lands. The cane internodes possess some special anatomical traits that could be used in inducing hardiness against lodging in cereals. It also possesses some drought tolerant anatomical traits which provide survival during dry seasons. Excellent phloem and xylem transport system and gradual thickening of fibrous band; vascular bundle distribution, shape and sizes are noted worthy features. Moreover thickening of ground parenchyma with the ages of cane contributes to keep the culms erect. The ratio of parenchyma, fiber and conducting tissue are ideal for food transport and food reserve.

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