Anatomy of Lignosus Bean (Dipogon lignosus) III. Stem

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Abstract: Anatomical investigation has been made on the stem of lignosus bean (Dipogon lignosus (L.) Verde.) at different stages of growth. The epidermis is single layered consisting of small and large cells. The epidermis bears multicellular hair and glandular trichomes. Beneath the epidermis there are 5-6 layers of cortical cells in the stem. The primary vascular tissue appears after the elongation of the first internode of the stem of lignosus bean. The internode between cotyledonary node and first leaf is considered as the first internode of the stem. The vascular bundles are collateral and arranged in a ring. The secretory cells devoid of tanniferous contents have been observed in the phloem region of the younger stem but not in older ones. The cambium initiates in the primary vascular bundle between xylem and phloem at the basal part of the stem of 7 days old plant. The cambium is at first confined to the fasicular region. Subsequently it extends into the interfascicular region forming a complete cambial ring. After the formation of fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. Some vessel members are paired while the others are solitary. Most of the paired vessels are radially arranged. The paired vessel members are more in number in the mature stem compared to that of the younger stem. The parenchyma covers the major area of the secondary xylem. The sclerenchymatous band adaxial to all large and small vascular bundles is discontinuous. The pith resembles a typical dicotyledonous stem. The phellogen appears in the deeper cortex and gives rise to 4-6 layers of cork cells abaxially and 3-4 layers of phellem axially.

Key words: Lignosus bean, Dipogon lignosus, anatomy, stem

Introduction
Anatomical features of the root and hypocotyl of lignosus bean (Dipogon lignosus (L.) Verde.) have been reported in the previous articles of the series (Bari and Prodhon, 2001; Prodhon and Bari, 2001). In this article the anatomy of the stem will be described so that a clear picture about the internal structures of the taxon may be obtained.

Materials and Methods
Mature seeds of lignosus bean (Dipogon lignosus (L.) Verde.) were collected from the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh. The experiment was carried out in the Bangladesh Agricultural University Farm as well as in the Department of Crop Botany during the study period between August, 1998 and June, 2000. The seeds were sown in polybags. The polybags were filled with thoroughly prepared soil of the plots. Some seedlings of the polybags were transplanted in the pits of the plots. The polybags were kept exposed to the normal weather conditions, so that the plants of both polybags and plots got more or less similar weather conditions (Bari and Prodhon, 2001; Haque and Prodhon, 1991; Prodhon and Bari, 2001; Prodhon and Haque, 1988). Some seeds were also placed on moist filter paper in large petri dishes in the laboratory at room temperature of about 25-28°C. The sprouting is considered as the zero hour of age of the plant (Bari and Prodhon, 2001; Prodhon and Bari, 2001).

From the fifth day of germination stems were collected till the plants were 18 days old. The samples were collected from both polybags and petri dishes, and were fixed in Craf III (Sass, 1958) after making small pieces of about 5 mm in length. Similarly samples of stem of 30, 60, 180 and 210 days old plants of the plots were also collected and fixed in FAA (Johansen, 1940). The materials fixed in Craf III and FAA were dehydrated through the tertiary butyl alcohol (TBA) series (Haque and Englemann, 1978; Haque and Prodhon, 1987; Prodhon and Bari, 2001; Prodhon and Haque, 1986). The materials fixed in FAA were washed in running tap water for 2-3 hrs before dehydration. The succulent materials were dehydrated gradually making more graces of alcohol to avoid severe shrinkage (Bari and Prodhon, 2001; Prodhon and Bari, 2001).

The dehydrated materials were gradually infiltrated with paraffin oil and low melting point (49-51°C) paraffin wax for 1-3 days (Haque and Prodhon, 1987; Prodhon and Bari, 2001; Prodhon and Haque, 1986). After infiltration the materials were embedded in high melting-point (61-63°C) paraffin wax. There was less shrinkage when the materials were infiltrated for a longer period. Serial transverse sections were made at 10 micron by a rotary microtome. The sections were stained with safranin and fast green and mounted in Canada balsam after proper dehydration with ethyl alcohol and clearing with xylene (Bari and Prodhon, 2001; Haque and Englemann, 1978; Johansen, 1940; Prodhon and Bari, 2001; Prodhon and Haque, 1986). Free hand sections were also made from fresh and fixed materials and temporary slides were made.

Results and Discussion
Epidermis: There is a single layer of epidermis in the stem of lignosus bean (Dipogon lignosus). The epidermis consists of small and large cells. Both small and large cells are irregularly distributed throughout the epidermis. The epidermal cells are more or less round, oval or polygonal in shape as seen in transverse sections of the stem of 9 days old plants. The epidermis bears multicellular hair and glandular trichomes. Similar types of hair and trichomes are also found in all aerial parts of the pigeonpea plants, except in petals and stamens (Bisen and Shealdrake, 1981). In Phaseolus the hair are glandular type and club-shaped with or without a distinct stalk (Metcalfe and Chalk, 1950). The abaxial, adaxial and lateral walls of the epidermal cells are more or less uniformly thick as seen in transverse sections of the middle and upper parts of the first internode of the stem of 9 days old plant (Fig. 3, 7). After considerable thickening of the abaxial wall a thin layer of cuticle is formed over the epidermis as seen in basal part of the stem of 9 days old plant (Fig. 3, 10). A thin cuticle has been observed in the stem of pigeonpea by Hossain (1999). The epidermal cell walls of the stem have been found to be thicker than those of the root (Prodhon and Bari, 2001). According to Essau (1965) the cell walls of the epidermis may
Fig. 1: T.S. of the upper part of 1st internode (epicotyl) of the stem of a 9 days old plant showing cambium (c), phloem (ph), xylem (x) and xylem vessels (v). X 520.

Fig. 3: T.S. of the upper part of 1st internode of the stem of a 9 days old plant showing epidermis (e) with cuticle and hair, cortex (cor), vascular bundles with bundle caps (discontinuous pericycle), cambium (c), xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). Cambium forms a ring except few ray regions where pith is continuous with the cortex. X 260.

Cortex: There are 6-8 layers of cortical cells in the stem of lignosus bean as seen in transverse sections (Fig. 3, 7, 10). There are large and small cells in cortex. The abaxial and adaxial cells of the cortex are smaller than that of the middle region. Most of the cells are round, oval or polygonal in shape while others are irregular. There are small intercellular spaces in the cortical parenchyma. With the age the intercellular spaces become prominent. The stem is not completely round but slightly ridged. In the mature stem there are small ridges and shallow furrows. The number of cortical layer is more in the ridge and less in the furrow (Haque and Prodhon, 1987; Metcalfe and Chalk, 1950). The number of cortical layer varies according to the age, size and level of secondary growth of the plant or plant parts.
Fig. 5: T.S. of the middle part of the 1st internode of the stem of a 7 days old plant showing epidermis (e) with cuticle and hair, cortex (cor), large and small vascular bundles, bundle caps or discontinuous pericycle (pc), cambium, xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). Epidermal circumference is wavy. X 280.

Fig. 7: T.S. of the middle part of the 1st internode of the stem of a 9 days old plant showing epidermis (e) with cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle (Pc), cambium (c), xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). The epidermal circumference is more or less smooth. Cambium forms a ring except few ray regions where pith is continuous with the cortex. X 260.

Fig. 6: T.S. of the middle part of the 1st internode (epicotyl) of the stem of a 7 days old plant showing epidermis (e) with cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle, cambium (c), xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). Epidermal circumference is wavy. Cambium forms a ring except few ray regions where pith is continuous with the cortex. X 275.

The cortical cells of the basal region of the stem have been found to be larger than the upper region as seen in transverse sections of 9 days old plant. The cortex exhibits morphological variations in its radial row of cells. In the basal parts of the stem, the cortical cells of the middle region are larger in size and comparatively thin walled with prominent intercellular spaces as seen in transverse sections of 9 days old plant (Fig.

Fig. 8: T.S. of the basal part of the stem of a 7 days old plant showing epidermis (e) with cuticle and hair, cortex (cor), large and small vascular bundles, discontinuous pericycle, cambium, xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). X 280.

9, 10). The abaxial layer of cortical cells are smaller in size and comparatively thick walled with small intercellular spaces. The adaxial layer is also smaller and thick walled having small intercellular spaces. At the upper part of the stem the abaxial 2-3 layers of cortical cells are smaller in size, comparatively thick walled with small intercellular spaces as seen in transverse sections of 9 days old plant (Fig. 3). There is little or no intercellular spaces between the outermost layer of the cortex and the epidermis.
Fig. 9: T.S. of the basal part of the stem of a 9 days old plant showing epidermis (e) with cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle, cambium (c), xylem (both primary and secondary), phloem (both primary and secondary) and pith (p). The epidermal circumference is smooth. Cambium forms a continuous ring. X 266.

Fig. 10: T.S. of the basal part of the stem of a 9 days old plant showing epidermis (e) with cuticle and hair, cortex (cor), vascular bundles, discontinuous pericycle (Pc), cambium (c), secondary xylem (sx), secondary phloem and pith (p). Cambium forms a continuous ring. X 260.

The outermost layer of the cortex contains chloroplasts. The adaxial one or two layers of cortical cells are smaller in size, comparatively thick walled having small intercellular spaces. No endodermis has been found in the stem of lignosus bean during present investigation. The cortical cells become tangentially flattened in the mature stem. The cortical cells of the stem of 9 days old plants have been found to be smaller than that of the hypocotyl (Bari and Prodhan, 2001). The cells of the innermost layer of the cortex have not been found to contain starch. A thorough developmental study is required to disclose its similarity to the endodermis or to the starch sheath. The tanniferous cells have not been found in the cortical region of the stem but have been observed in the cortex of the younger hypocotyl (Bari and Prodhan, 2001). Sarkar (1996) has reported tannin cells in the cortex of the stem of Sesbania rostrata and Sesbania sesban. The abaxial cells of the cortex are ruptured and broken here and there and disorganized in the later stage of growth.

Primary vascular tissue: The primary vascular tissue appears after the elongation of the first internode of the stem. It forms for a short period as the cambium appears soon. The internode between cotyledonary node and first leaf is considered as the first internode of the stem. The arrangement of vascular tissues is collateral as seen in transverse sections in the first internode of the stem of 9 days old plant (Fig. 2, 7, 10). The...
Fig. 13: T.S. of the stem of a mature (210 days old) plant showing epidermis (e) with cuticle, cortex (cor), cambium (c), secondary xylem (sx), xylem vessel (v), fibers, ray and axial parenchyma, secondary phloem (sp) and pith (p). A few secondary xylem vessels remain in paired. X270.

The vascular bundles are arranged in a ring. There are two types of vascular bundles, large and small, having one or more small vascular bundles in between two large bundles. The sclerenchymatous cells are present on the abaxial side of all large and small vascular bundles. It is pericycle. The pericycle is discontinuous in the stem of lignosus bean and is also found in hypocotyl and root of lignosus bean (Bari and Prodhani, 2001; Prodhani and Bari, 2001). Similar result has been reported for the stem of pheonpea (Hossain, 1999).

Radially each group of sclerenchyma consists of more or less 2-8 cells. Two adjacent groups are connected by one or two layers of sclerenchyma. Sometimes 2-3 or more vascular bundles, either large or small, contain a single band of sclerenchyma on their abaxial sides. The cells of the pericycle contain prominent secondary thickening and large lumen. A continuous ring of sclerenchyma constituting the pericycle has been reported for pheonpea stem (Bisen and Sheidrake, 1981). Such sclerenchymatous cells have different origin in different plants (Essau, 1977). So, the origin of this sclerenchymatous tissue of lignosus bean needs a thorough investigation.

Primary xylem: The large vascular bundle contains xylem and phloem but the small vascular bundle may or may not contain both xylem and phloem. The primary xylem develops only for a short period. Many vessel members are present in the large vascular bundle but a few vessel members have been found in the small vascular bundle. The vessels are small and large as seen in transverse sections of the stem of 9 days old plant (Fig. 2, 10). The size of the cells have been considered by diameter and not by length. Most of the vessel members are radially arranged while the others are scattered in the vascular bundle. The smaller vessels are adaxial to the bigger ones. Sometimes the small and large vessels have been found to remain side by side as seen in transverse sections of the stem of 9 days old plant (Fig. 2, 10). The mature vessel is completely devoid of protoplasm and contains thick secondary wall with large lumen.

The vessel members are round, oval or polygonal in shape as seen in transverse sections. There are 4-9 vessel members in large vascular bundle and 1-4 vessel members in small vascular bundle. Some vessel members are paired while the others are solitary. The paired vessel members are more in number in the mature stem as compared to the younger stem (Fig. 8, 11, 13). Some individual tracheary elements have also been found to differentiate in between two groups of xylem. The cells in between and around the vessel members are mostly of axial xylem parenchyma, other elements are primary xylem fibers. The primary tracheary elements are continued to form in the steam of lignosus bean till the activity of the cambium continues. No secondary growth has been observed in the stem till the plant is 9 days old.

Primary phloem: There are several poles of primary phloem adaxial to the primary xylem. In the large bundle there are a number of sieve elements and phloem parenchyma in the phloem zone. Like primary xylem they mature rapidly. There may be only one mature or immature sieve element in small vascular bundle. There are several poles of sieve elements in the younger stem. The number of phloem poles increases along with the age. The sieve elements are separated from each other by phloem parenchyma. Each pole consists of 1-4 sieve tube elements. In many cases sieve tubes have been found to accompany their companion cells. The sieve elements are also found to be scattered as seen in transverse sections of the stem of 9 days old plant (Fig. 3, 10). Some of the sieve elements are well apart and some are closer to each other. The primary sieve elements are continued to form in the stem till the activity of cambium continues. No secondary growth has been observed in the first internode of the stem till the plant becomes 9 days old. The secretory cells devoid of tanniferous contents, have been observed in the phloem region of younger stem but not in the older ones of lignosus bean during present investigation. The tannin cells have been found within and just inside the primary phloem in the hypocotyl and root of Phaseolus mungo (Haque and Engleman, 1978). Compton (1912) and Dow (1932) have reported tannin sacs in the stem and hypocotyl but not in the root of Phaseolus and other legumes. However, tannin cells have been found in the stem and hypocotyl but not in the root of lignosus bean (Bari and Prodhani, 2001; Prodhani and Bari, 2001). Secretory cells or ducts have been reported in the phloem region at the apical part of the stem of Sesbania formosa (Hossain, 1997).

Cambium: The cambium has been found to initiate in the primary vascular bundle between xylem and phloem at the basal part of the first internode of the stem of 7 days old plant. Gradually it extends towards the upper part. The cambial activity has been observed in the vascular bundle of the stem of 9 days old plant. Soon after the formation of the fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. The fascicular cambia in both large and small vascular bundles have been found to form simultaneously. The cambium is at first confined to the fascicular region of the primary vascular tissue. Subsequently it extends into the interfascicular region forming a complete cambial ring. Similar results have been reported by Metcalfe and Chalk (1950) and Haque and Prodhani, (1987, 1991). The cambium forms a continuous ring in the first internode of the stem of 9 days old plant excepting a few regions where cortical tissue has been found to be continuous with the pith (Fig. 3). The cambial ring becomes complete later on. After the formation of the cambial ring, the cambium becomes active at all points of the ring. The cambium is active in both abaxial and adaxial sides but it is more active on its adaxial
side. In the active state of growth, the cambial zone consists of 5-6 layers of cells of cambial initials and their derivatives. The cells in the cambial zone are tangentially flattened and compact. The initials and the derivatives of the cambium in the stem have not been closely observed during present investigation. The cambial activity seems to be more in the stem compared to that in the root (Prodhon and Bari, 2001). The cambium has been found to remain active up to the maturation or senescence of the plant.

Secondary xylem: The secondary xylem has been found to form adaxially in the fascicular region after the formation of fascicular cambium at the basal part of the stem of 9 days old plant. Gradually it extends towards the upper part of the stem as seen in transverse sections. It appears that the cambium becomes active in the fascicular region earlier than in the interfascicular region. So the secondary tissues are formed in the fascicular region much earlier than those in the interfascicular region. The fascicular cambium begins to form secondary tissues while the elements of primary vascular tissues are differentiating. After a level of secondary growth in the fascicular region, interfascicular cambium is formed and gives rise to secondary tissues. Mettrafe and Chalk (1950) have reported that the cambium is at first confined to the primary vascular bundles and subsequently it extends into the interfascicular region and gives rise to secondary tissues there. Similar result has been reported for Brassica campestris (Haque and Prodhon, 1987, 1991). So at the early stage, the secondary growth is more in the fascicular region compared to that of the interfascicular region. Ultimately the secondary tissues become more or less equal in both the regions due to the vigorous activities of the interfascicular cambium. After the formation of cambial ring the differentiation of secondary xylem takes place rapidly. The cambium gives rise to tracheary elements, xylem fibers and xylem parenchyma (both ray and axial).

The secondary xylem vessel begins to form adaxial to the cambium as seen in the basal part of the stem of 9 days old plant (Fig. 9, 10). The secondary xylem vessel is also found to form at middle and upper part of the first internode of the stem of 9 days old plant (Fig. 3, 7). No secondary xylem has been found to form in the interfascicular region where no cambium has yet been formed as seen in the middle and upper part of the first internode of the stem of 9 days old plant. Some vessels are large and some are small as seen in transverse sections of 9 days old plant (Fig. 1, 7, 10). In the mature stem, some vessels are several times larger than the rest of the secondary xylem vessels as well as primary xylem vessels. The size of the vessel is considered by diameter and not by length during the present investigation. Most of the vessels are arranged radially and the others are distributed randomly as seen in transverse sections of young and mature stems. Some small vessels are arranged in between or among the big vessels while other small vessels are distributed randomly throughout the xylem region. Some of the vessels are found in pairs while the others are solitary. Most of the paired vessels are radially arranged. Both large and small vessels are round, oval or polygonal in shape while a few vessels are irregular in shape. The walls of both small and large vessels are thick and lignified. The mature vessels are devoid of protoplasm with prominent secondary thickening as seen in transverse sections. The walls of the newly formed vessels are very thin and irregular in shape. The spaces between the secondary xylem vessels are filled with other elements of secondary xylem like ray, axial parenchyma and fibers. The axial parenchymatous cells have been found in between and around the vessel members. They are thick walled. The ray cells are smaller in size. Most of the ray cells in the xylem are uniseriate, few are biseriate and some are multiseriate and become gradually thickened. The ray cells are less thickened compared to that of the axial xylem parenchyma of secondary origin. The parenchyma covers the major area of the secondary xylem. The fibre cells are limited in number. The fibre cells are highly lignified, thick walled with small lumen. They are mostly pentagonal, hexagonal or square in shape as seen in transverse sections. The number of layers of secondary xylem increases in the stem along with the age of the plant.

Lots of secondary xylem are found in the stem of mature plant. The stem at the flowering stage of the plant contains secondary xylem of considerable thickness. Different components of secondary xylem have not been studied thoroughly during present investigation. Some attention has, however, been given on the vessel members, axial and ray parenchyma and fibers. With growth and maturity secondary xylem pushes the primary xylem into the center. The elements of primary xylem both proto and meta remain intact bordering the pith as seen in transverse sections of the mature stem (Fig. 12, 13).

Secondary phloem: The secondary phloem has been found to form adaxially in the fascicular region after the formation of fascicular cambium at the basal part of the stem of 9 days old plant. Gradually it extends towards the upper part of the stem as seen in transverse sections. It appears that the cambium becomes active in the fascicular region earlier than in the interfascicular region. So the secondary tissues are formed in the fascicular region much earlier than those in the interfascicular region. The fascicular cambium begins to form secondary tissues while the elements of primary vascular tissues are differentiating. After a level of secondary growth in the fascicular region, interfascicular cambium is formed and gives rise to the secondary tissues. According to Mettrafe and Chalk (1950) the cambium is at first confined to the primary vascular bundles and subsequently it extends into the interfascicular zone and gives rise to secondary tissues there. Similar results have been reported for Brassica campestris (Haque and Prodhon, 1987, 1991). So at the early stage, the secondary growth is more in the fascicular region than that in the interfascicular region.

In the large vascular bundle there are a number of sieve elements and phloem parenchyma in the phloem zone. They mature rapidly like primary xylem. In the small vascular bundle there may be one or two mature or immature sieve elements. Before maturation of the sieve elements cambium becomes active and gives rise to secondary phloem. It is difficult to distinguish primary sieve elements from secondary ones and it is difficult to distinguish primary sieve elements from secondary ones because these are more or less of same size and shape. Some sieve elements with their companion cells and axial phloem parenchyma have been found to form in the stem of 9 days old plant (Fig. 7, 9). There are several pairs of sieve elements in big vascular bundle. In one pole of sieve elements, there may be one or more sieve tube elements. The phloem zone is
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narrow. Radially it consists of 4-6 layers of cells in the big vascular bundle and 3-4 layers of cells in the small vascular bundle as seen in the stem of 9 days old plant (Fig. 2, 7, 9). The thickness increases up to 7-10 layers as seen in the mature stem (Fig. 11, 12, 13). A number of parenchyma, both axial and ray, of secondary origin is present in the phloem zone. The ray cells are large as seen in transverse sections. The secondary sieve elements become narrow along with the age. The fibre cells have not been observed in secondary phloem during the present investigation. The secondary phloem continues to form and the sieve elements remain active till the maturity or senescence of the plant.

Pith: The pith is prominent and the cells are thin walled with prominent intercellular spaces. It is composed of small and large parenchymatous cells. The central cells of the pith are large while the abaxial cells are smaller in size. The pith cells are round, oval or somewhat polygonal in shape as seen in transverse sections (Fig. 6, 11). In primary structure of the stem, the pith increases in size due to the increase in diameter of the pith cells as well as the size of the intercellular spaces. The pith gradually decreases in size due to the continuous addition of secondary xylem. Similar findings have been reported for Brassica (Haque and Prodhan, 1987, 1991; Metcalf and Chalk, 1950). Three to six layers of small and moderately thick walled parenchymatous cells constitute the periphery while a number of comparatively large and thin walled cells compose the center of the pith of the older stem. Due to the stress of axial growth of the stem and the addition of secondary xylem towards the center, the peripheral pith cells lose their intercellular spaces and become narrow (Esau, 1965) but the pith cells remain more or less unaffected at the center.

Periderm: The well developed periderm has been observed in the basal part of the stem of lignous bean. The phellogen has been found to initiate from the deeper cortex and gives rise to cork cells abaxially and phellem adaxially as revealed from the transverse sections in the basal part of the stem. The number of cork cells in a radial row depends on the age and size of the plant parts (Esau, 1965). In Sesbania of Papilionaceae, a well developed periderm has been reported by Hossain (1997) and Sarker (1998). The development and morphology of different components of periderm have not been studied during present investigation. However, the phellogen in the stem of lignous bean has been found to be similar in origin, development, structure and pattern as that of the root and hypocotyl (Barin and Prodhan, 2001; Prodhan and Bari, 2001). The origin, development and activity of phellogen have been reported for Aesculus and Robinia by Arzee et al. (1968, 1970) and Waisel et al. (1967). During the present investigation, the periderm consists of 4-5 layers of cork cells and 3-4 layers of phellem with a narrow zone of differentiating phellogen. The periderm develops one after another from deeper cortex and as a result cortex is eliminated due to the formation of periderm. Similar results have been reported for Sesbania (Hossain, 1997; Sarker, 1996). Generally the periderm is formed in the outer part of the cortical region of the stem (Esau, 1965; Haque and Hossain, 1978).

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