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Anatomy of Lignosus Bean *(Dipogon lignosus)* II: Hypocotyl

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Abstract: Anatomical investigation has been made on the hypocotyl of lignosus bean *(Dipogon lignosus* (L.) Verde.) at different stages of growth. The hypocotyl is like stem in structure. The epidermis is single layered with large and small cells. Beneath the epidermis there are 9-12 layers of cortical cells. The vascular bundles are collateral and arranged in a ring. There are two types of vascular bundles, large and small. Lots of tanniniferous cells have been found in the cortex and primary phloem region of the younger hypocotyl but not in older ones. Adaxial to the epidermis there are 5-7 layers of lacunar collenchyma at the younger hypocotyl. Along with the age the lacunar collenchyma cells have been replaced by the parenchyma cells. The cambium initiates in the primary vascular bundles between xylem and phloem at the basal part of hypocotyl of 3 days old seedlings. The cambium is at first confined to the fascicular region. Subsequently it extends into the interfascicular region and forms a complete cambial ring. The sclerenchymatous band known as pericycle is discontinuous. Two adjacent groups of sclerenchyma are connected by one or two layers of sclerenchyma cells. Sometimes 3-4 vascular bundles, either large or small, contain a single band of sclerenchymatous cells on their abaxial sides. The phellogen appears in the cortex and gives rise to 4-6 layers of cork cells abaxially and 2-4 layers of phelloderm adaxially.

Key words: Lignosus bean, *Dipogon lignosus,* anatomy, hypocotyl

Introduction

Anatomical features of the root of lignosus bean *{Dipogon lignosus* (L.) Verde.) have been reported in the first article of the series (Prodhan and Bari, 2001). In this article the anatomy of the hypocotyl 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.) Yerdc.) were collected from the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh. The experiment was carried out in Bangladesh Agricultural University Farm as well as in the Department of Crop Botany during the study period between August, 1999 and June, 2000. The seeds were sown in polybags. The polybags were filled up 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 condition, so that the plants of both polybags and plots got more or less similar weather conditions (Haque and Prodhan, 1991; Prodhan and Ban, 2001; Prodhan and Haque, 1986). Some seeds were also placed on moist filter paper in large petri dishes in the laboratory at room temperature of about 26-28(°)C. The petri dishes were kept in the dark for about 24 hours. The sprouting is considered as the zero hour age of the plant (Prodhan and Bari, 2001).

From the third day of germination hypocotyls were collected every day from the plants till they were 15 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 hypocotyl of 20, 30, 50, 180 and 210 days old plants of the plots were also collected and fixed in FAA. The materials fixed in Craf IU and FAA were dehydrated through the tertiary butyl alcohol (TBA) series (Haque and Engleman, 1978; Haque and Prodhan, 1987; Prodhan 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 grades of alcohol to avoid severe shrinkage (Prodhan and Ban, 2001).

The dehydrated materials were gradually infiltrated with paraffin oil and low melting point (51 °C) paraffin wax for 1-3 days (Haque and Prodhan, 1987; Prodhan and Haque, 1986). After infiltration the materials were embedded in high-melting-point (61°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 (Haque and Engleman, 1978; Johansen, 1940; Prodhan and Haque, 1986). Freehand 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 hypocotyl of lignosus bean *(Dipogon lignosus).* The epidermis consists of small and large cells. At the young hypocotyl the epidermal cells are thin walled and regularly arranged. With the age the cells become irregular. The epidermal cells have been found to be regular in shape, size and arrangement as seen in transverse sections of the hypocotyl of 3 days old seedlings. The circumference of the epidermis is more or less smooth. The cells are square, oval, round or rectangular in shape as seen in transverse sections of the hypocotyl of 5 days old seedlings (Fig. 1). With the age the shape gradually become more or less oval, round, ovate, obovate, pentagonal or slightly rectangular as seen in the 7 or 9 days old seedlings (Fig. 2,4, 6). The cells maintain more or less similar shape as seen in transverse sections of the hypocotyl of more aged plants.

The abaxial, adaxial and lateral walls of epidermis of hypocotyl up to 5 days of germination have been found to be almost equally thin. With the age the walls gradually become thick. The thickening initiates in abaxial wall and gradually it extends to lateral walls and ultimately to the adaxial walls as seen in transverse sections of the hypocotyl of 5, 7 and 9 days old seedlings. The cuticle appears over the abaxial wall of the epidermis of 7 days old seedlings (Fig. 2,4). Gradually it thickens. The epidermis bears multicellular hair or glandular trichomes.

Cortex: There are 9-12 layers of cortical cells in the hypocotyl of lignosus bean *((Dipogon lignosus).* The cortical cells are of

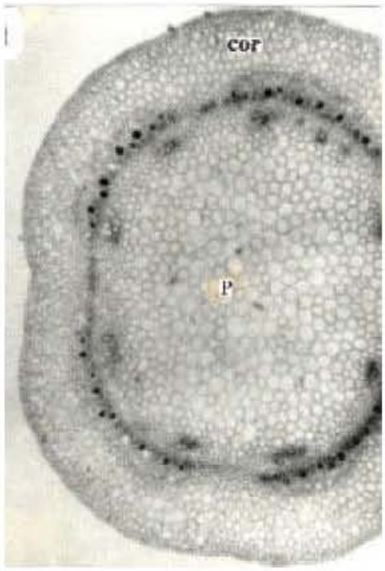

Fig. 1: T.S. of the upper part of the hypocotyl of a 5 days old plant showing epidermis, cortex (cor), vascular bundles and pith (p). Lots of tannin cells are present in the primary phloem region. X 132.

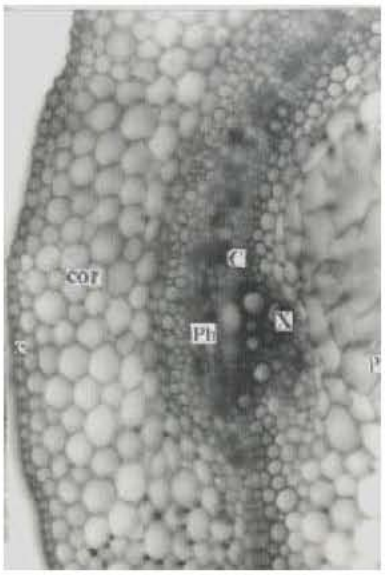

Fig. 3: T.S. of the middle part of the hypocotyl of a 9 days old plant showing epidermis (e), cortex (cor), cambium (c), xylem (x), phloem (ph) and pith (p). X 260.

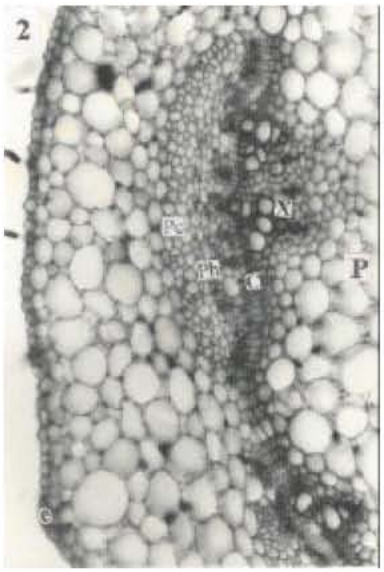

Fig. 2: T.S. of the upper part of the hypocotyl of a 7 days old plant showing epidermis (e) with hairs, cortex (cor), discontinuous pericycle (Pc), cambium (c), xylem (x), phloem (ph) and pith (p). X 260.

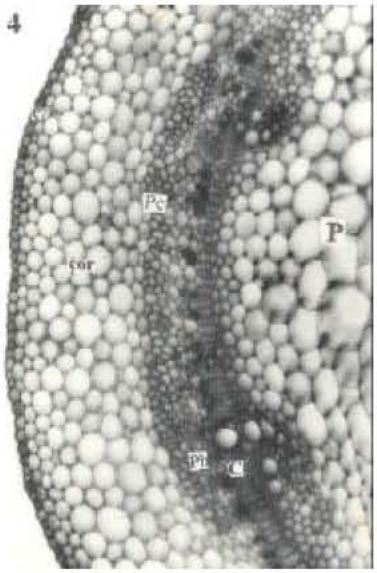

Fig. 4: T.S. of the middle part of the hypocotyl of a 9 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle (Pc), cambium (c), xylem (x), phloem (ph) and pith (p).X260.

two types, collenchyma and parenchyma. Adaxial to the epidermis there are 5-7 layers of lacunar collenchyma as seen in transverse sections of the hypocotyl of 3 days old seedlings. Adaxial to the collenchyma there are 4-5 layers of parenchymatous cells in the 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, some are hexagonal or pentagonal while the others are irregular (Fig. 4, 6). In the cortical parenchyma there are small intercellular spaces that become prominent with the age. The intercellular spaces also increases in number. The cortical cells of the hypocotyl seem to be smaller than those of the root (Prodhan and Ban, 2001). Similar reports are available (Esau, 1965; Haque and Prodhan, 1991; Prodhan and Haque, 1986). The

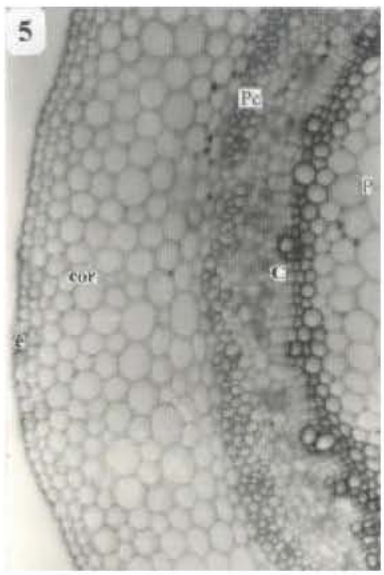

Fig. 5: T.S. of the lower part of hypocotyl of a 7 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle (Pc), cambium (c), xylem, phloem and pith (p). X 260.

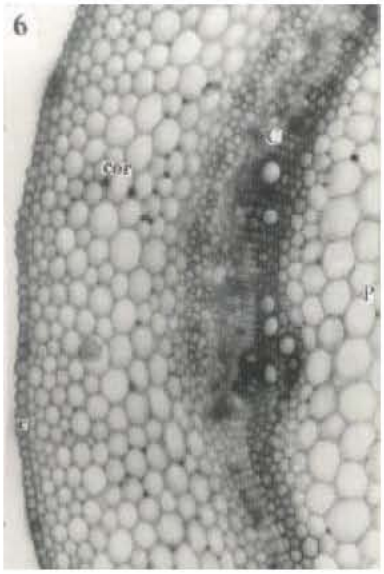

Fig. 6: T.S. of the lower part of hypocotyl of a 9 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle, cambium (c), xylem, phloem and pith (p). X 260.

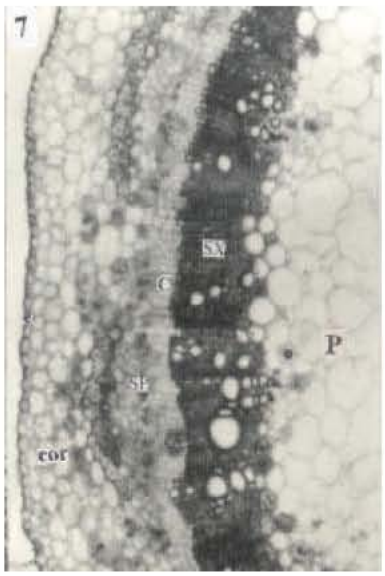

Fig. 7: T.S. of the lower part of hypocotyl of a 50 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle, cambium (c), secondary xylem (sx), secondary phloem (sp) and pith (p). X 260.

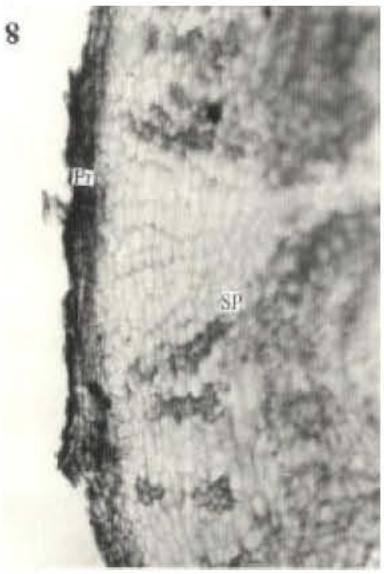

Fig. 8: T.S. of the hypocotyl of a 210 days old (mature) plant showing secondary phloem (sp) and periderm (Pr) with cork cells, phellogen and phelloderm. X 280.

cells of the outermost layer of cortex are more or less similar to the cells of epidermis specially at the base of younger hypocotyl. The cortical cells of the apical region of the hypocotyl have been found to be larger than the basal region as seen in transverse sections of 9 days old seedlings. 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 lacunar collenchyma decreases with the age. The lacunar collenchyma cells have been replaced by the parenchyma cells as seen in the hypocotyl of 9 days old seedling. The tannin cells have been observed in the cortex of the hypocotyl of 3-5 days old seedlings of lignosus bean

during present investigation. The tanniniferous cells have not been observed in the older hypocotyl. No tanniniferous cells have been found in the cortex of the root of lignosus bean (Prodhan and Bari, 2001). 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 arrangement of vascular tissues is collateral in the upper part of the hypocotyl as seen in transverse sections of 5 days old seedling (Fig. 1). Similar arrangement is also observed in the middle and basal part of the hypocotyl at the young stage. The vascular bundles are arranged in a ring. There are two types of vascular bundles, large and small. There are one, two or three 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 hypocotyl of lignosus bean. Radially each group of sclerenchyma consists of more or less 2-5 cells. Two adjacent groups are connected by one or two layers of sclerenchyma. Sometimes 3-4 vascular bundles, either large or small, contain a single band of sclerenchymatous cells on their abaxial sides. The sclerenchymatous cells contain prominent secondary thickening and large lumen. A continuous ring of sclerenchymatous fibers constituting the pericycle has been reported for pigeonpea stem (Bisen and Sheldrake, 1981). Such sclerenchymatous fibers have different origin in different plants (Esau, 1965, 1977). Therefore, the origin of this sclerenchyma tissue of lignosus bean needs a thorough investigation.

Primary xylem: The large vascular bundle contains xylem and phloem but the small bundle may or may not contain both xylem and phloem. There are several poles of primary phloem outside the primary xylem. The primary xylem develops only for a very short period. In the large vascular bundle many vessel members are present but in the small vascular bundle only a few vessel members have been found. There are two types of vessel members, large and small as seen in transverse sections of the hypocotyl of 5 days old seedling (Fig. 1). In the vascular bundle most of the vessels are radially arranged, others are scattered. The smaller vessels are adaxial to the bigger ones. The small and large vessels have been found to remain side by side as seen in transverse sections of 5 days old seedling. The mature vessel contains thick secondary wall and large lumen. It is completely devoid of protoplasm.

The vessels are round, oval or polygonal in shape as seen in transverse sections. In the upper part of hypocotyl there are 4-8 vessel members in large vascular bundle and 1-4 vessel members in small vascular bundle. Some individual tracheary elements have also been found to differentiate in between two groups of xylem. The primary tracheary elements are continued to form in hypocotyl till the activity of the cambium continues. No secondary growth has been observed in the basal part of hypocotyl till it is 7 days old.

Primary phloem: In the large vascular bundle there are a number of sieve elements and phloem parenchyma in the phloem zone. Like primary xylem they mature rapidly. In the small vascular bundle there may be only one mature or immature sieve element. There are several poles of sieve elements as seen in the upper part of hypocotyl of 5 days old seedling. 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 hypocotyl of 5,6 or 7 days old seedlings. They are well aparted, some ones are closer and some are aparted. The primary sieve elements are continued to form in hypocotyl till the plants are 7 days old after which secondary sieve elements begin to form.

Lots of tanniniferous cells have been found to the primary phloem region adaxial to the individual sclerenchymatous band of the pericycle in the upper part of the hypocotyl of 5 days old seedlings (Fig. 1). The tanniniferous cells have been observed in the younger hypocotyl but not in the older ones of the lignosus bean during present investigation. Rows of tannin cells have been found within and just inside the primary phloem in the hypocotyl-root axis of young seedlings of *Phaseolus mimgo* (Haque and Engleman, 1978). These are large and conspicuous cells in the hypocotyl and root (Haque and Engleman, 1978). Doutt (1932) have also 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 primary phloem region of the hypocotyl but not in the root of lignosus bean (Prodhan and Bari, 2001).

Cambium: The cambium has been found to initiate in the basal part of the hypocotyl of 3 days old seedling. Lining up of the cells in the vascular bundle between xylem and phloem, and the tangential divisions of these cells show the initiation of the cambium. The cambium is confined only in the fascicular region at this age. The fascicular cambia in both large and small vascular bundles have been found to form simultaneously. The interfascicular cambium appears later on. Metcaife and Chalk (1950) have reported that the cambium is at first confined to the primary vascular bundles but subsequently it extends into the interfascicular regions. After the formation of the fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. No cambium has been observed in the middle and upper part of the hypocotyl at the same age. Although the cambium initiates in the basal part of the hypocotyl of 3 days old seedling, no activity has been observed till the plant is 5 days old. The cambium forms a continuous ring in the basal part of the hypocotyl of 5 days old seedling excepting few places of interfascicular regions where cortical tissue has been found to be continuous with the pith. The cambial ring becomes complete later on. A complete ring has been found in the basal part of the hypocotyl of 7 days old seedling (Fig. 5). A continuous cambial ring has also been observed in the middle and upper part of the hypocotyl of 9 days old seedling (Fig. 4). The cambium consists of several layers of tangentially flattened cells. It has been found to remain active up to the maturation/senescence of the plant.

Secondary xylem: The secondary xylem begins to form in the fascicular region adaxially after the formation of the fascicular cambium in the basal part of the hypocotyl of 5 days old seedling. Gradually it extends towards the upper part of the hypocotyl as seen in transverse sections of 7 and 9 days old seedlings (Fig. 2). The cambium appears and becomes active in the fascicular region earlier than the interfascicular region.

So the secondary tissues are formed much earlier in the fascicular region 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 cambia are formed and give rise to secondary tissues. According to Metcaife and Chalk (1950) the cambium is at first confined to primary vascular bundles but later on it extends into the interfascicular zone and gives rise to the secondary tissues there. So at the early stage the secondary growth is more in the fascicular region than that of the interfascicular region. The secondary tissues ultimately become more or less equal in both the regions due to vigorous activities of the interfascicular cambium.

The secondary xylem vessels begin to form adaxial to the cambium as seen in the basal part of the hypocotyl of 7 days old seedling (Fig. 5). The secondary xylem vessels are also observed at the middle and upper part of the hypocotyl of 9 days old seedling (Fig. 3). No secondary xylem has been found to form in the interfascicular region where no cambium has yet been formed as seen in the upper part of the hypocotyl of 9 days old plant. The secondary xylem vessels are found to be large, medium and small in size. Some of the secondary xylem vessels are very large in size with big lumen compared to that of the primary xylem vessels. The vessel members are mostly round, oval, polygonal or hexagonal and others are irregular in shape as seen in transverse sections. The mature vessels are devoid of protoplasm with prominent secondary thickening. The number of layers of secondary xylem increases in the hypocotyl along with the age of the plant. Lots of secondary xylem are found in the upper part of the hypocotyl of 50 days old plant (Fig. 7). The hypocotyl at the flowering stage of the plant contains secondary xylem of considerable thickness. Different components of secondary xylem have not been studied during present investigation. Some attention has, however, been given on the vessel members, axial and ray parenchyma and fibers. Some of the secondary xylem vessels are arranged more or less radially and others are scattered as seen in transverse sections. Some vessels are arranged in group and some are solitary. The spaces between the secondary xylem vessels are filled up with other elements of secondary xylem like ray, axial parenchyma and fibers. The axial xylem parenchymatous cells have been found in between and around the vessel members. They are thick walled. The ray cells are small, uniseriate or multiseriate and become gradually thickened. The ray cells are less thickened than the axial xylem parenchyma of secondary origin. Most of the cells of secondary xylem in the hypocotyl are fibers. The fibers are highly lignified, thick walled with small lumen. They are mostly hexagonal or pentagonal in shape. 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 hypocotyl.

Secondary phloem: The secondary phloem begins to form in the fascicular region abaxially after the formation of the fascicular cambium in the basal part of the hypocotyl of 5 days old seedling. The secondary phloem gradually extends towards the upper part of the hypocotyl. Both primary and secondary sieve elements are well distributed outside the cambial ring. Some sieve elements with their companion cells, axial phloem parenchyma have been found to form in the hypocotyl of 7-9 days old plants. There are a number of sieve elements and phloem parenchyma in the phloem zone of large vascular bundle. Like primary xylem they mature rapidly. There may be only one mature or immature sieve element in the small vascular bundle. Before maturation of the sieve elements cambium becomes active and gives rise to secondary phloem abaxially. In one pole of sieve elements, there may be one or more sieve tube elements. It is difficult to distinguish primary sieve elements from the secondary sieve elements. The diameters of meta sieve tube elements and the secondary sieve tube elements are also same as seen in transverse sections of the hypocotyl of 9 days old plant. So by size one can not easily recognize primary and secondary sieve elements at this stage of growth.

Some phloem poles are present even where there are no corresponding xylem. There is a group of sieve elements in large vascular bundle as seen in 5 days old hypocotyl (Fig. 1). There are 3-6 sieve tube elements in large vascular bundle and 1-3 sieve tube elements in the small vascular bundle. The phloem zone is narrow. Radially it consists of 4-6 layers of cells as seen in the hypocotyl of 9 days old plant. The thickness increases up to 7-9 layers as seen in the hypocotyl of 50 days old plant (Fig. 7). Both axial and ray parenchyma of secondary origin are also limited in number. The ray cells are large as seen in transverse sections. Some hyperchromatic phloem parenchyma have also been observed in the phloem region. The secondary sieve elements become narrow along with the age. The fibre cells have not been observed in the secondary phloem during the present investigation. The secondary phloem continues to form and the sieve elements remain active till the maturity/senescence of the plant.

Pith: The pith is prominent in the hypocotyl. The pith cells are thin walled having intercellular spaces. The pith is composed of small and large parenchymatous cells. The cells are round, oval or somewhat polygonal in shape as seen in transverse sections (Fig. 6, 7). The pith increases in size due to increase in diameter of the pith cells as well as the size of the intercellular spaces. On the continuous addition of secondary xylem the pith gradually decreases in size. Similar finding has been reported for *Brassica campestris* (Haque and Prodhan, 1987, 1991). Two to three 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 older hypocotyl. Due to the stress of axial growth of the hypocotyl 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 at the center remain more or less unaffected. In the mature plant, the pith of the hypocotyl becomes small due to the secondary growth.

Periderm: The periderm normally forms in the hypocotyl of lignosus bean *(Dipogon lignosus)*. The periderm has been found to form in a circle. Due to the stress of secondary growth the epidermis ruptures here and there and the cells become partly or wholly disorganized. After the disintegration of the epidermis the phellogen develops. The phellogen has been found to initiate from the deeper cortex and gives rise to cork cells abaxially and phelloderm adaxially as revealed from the transverse sections of the hypocotyl (Fig. 8). The number of cork cells in a radial row depends on the age and size of the plant or plant parts. The initiation of phellogen and the development and morphology of different components of periderm have not been studied thoroughly during present investigation. The phellogen produces 4-6 layers of cork cells abaxially and 2-4 layers of phelloderm adaxially. The cork cells are apparently devoid of protoplasm and thick walled. They are tangentially flattened and brick shaped in appearance as

seen in transverse sections. The cells of phelloderm resemble cortical cells but they are smaller in size. The development of periderm in the hypocotyl of lignosus bean needs further investigation. The origin, development and activity of phellogen have been reported for many plants (Arzee *et al.*, 1970; Esau, 1965; Haque and Hossain, 1978; Haque and Prodhan, 1991).

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