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## Anatomy of the Hypocotyl of Country Bean

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**Abstract:** Anatomical investigation has been made on the hypocotyl of country bean (*Lablab purpureus* (L.) Sweet) at different stages of growth following the standard paraffin method of microtechnique. The basal part of the hypocotyl is root-like in structure while the middle and upper parts are stem-like. The transition of vascular tissues occurs in the basal part of the hypocotyl. The vascular bundles are collateral in arrangement in the middle and upper parts and radial in the basal part. There are two types of vascular bundles, small and large in the middle and upper parts. There are 1-2 small vascular bundles in between two large bundles. The large vascular bundle contains xylem and phloem but small bundle may or may not contain both xylem and phloem. There are several poles of primary phloem outside the primary xylem. In the basal part of the hypocotyl there are 4 strands of xylem alternating with phloem zones. Each xylem strand is divided into 2 xylem poles which are either connected or separated. There are several poles of phloem in the phloem region in between 2 xylem strands. The vessels in the xylem strands are found to be radially arranged as seen in the basal part of the hypocotyl. Lots of tanniferous cells with or without content have been found in the primary phloem region. The pericycle is discontinuous. Two adjacent groups of sclerenchyma are connected by one or two layers of sclerenchyma cells. Sometimes 1-2 vascular bundles, either large or small, contain a single band of sclerenchyma on their abaxial sides. The cambium appears and becomes active in the fascicular region earlier than the interfascicular region. The cambium appears in the basal part of the hypocotyl of 4 days old seedling. Gradually it extends towards the upper part. The cambium forms a ring in the basal part of the hypocotyl of 7 days old seedling. The epidermis, cortex and pith resemble a typical dicotyledonous plant. The phellogen appears in the cortex and gives rise to cork and phelloderm. A well developed periderm is formed in the hypocotyl of mature plant.

**Key words:** Country bean, *Lablab purpureus*, anatomy, hypocotyl

### INTRODUCTION

Country bean (*Lablab purpureus* (L.) Sweet (Syn. *Dolichos lablab* L., *D. purpureus* L., *Lablab niger* Medik)  $2n = 24$ ) is one of the leading winter vegetables in Bangladesh. It belongs to the sub-family Papilionaceae under the family Leguminosae. Internationally the crop is known by various other names such as hyacinth bean, field bean, seim bean, Dolichos bean or Indian bean<sup>[1,2]</sup>. There are 50 species of country bean (hyacinth bean) distributed throughout the world specially in the tropical and sub-tropical regions of Asia, Africa, America and Australia<sup>[3]</sup>. It is a self-pollinated crop and probably originated in India. Many types and forms of this crop are grown throughout Bangladesh. The cultivation of this crop is limited to mostly in homestead areas. It is a short live creeping perennial but used as an annual legume. It is used in different ways. Green pods are cooked as vegetable while dry seeds are eaten directly by frying or

cooking as "dal" and are also used in various preparations. Nutritionally its green edible pods provide about 25% protein (on dry wt. basis), vitamins such as vitamin A, vitamin C, riboflavin and minerals like magnesium, calcium, phosphorus, iron, sulphur and sodium<sup>[4,5]</sup>.

The biological phenomena of country bean plant have not been investigated thoroughly. Some sporadic works have been carried out with country bean plant at home and abroad. The morphological, physiological and physio-ecological features of different types of country bean are known to some extent but information on anatomical features of these plants is very limited. Available literature shows that some anatomical works so far have been done with *Dipogon lignosus*<sup>[6]</sup> and *Lablab purpureus*<sup>[7]</sup>. The development and structure of different tissues such as cowpea<sup>[8]</sup>, *Sesbania rostrata*<sup>[9]</sup>, *S. sesban*<sup>[10]</sup>, *S. formosa*<sup>[11]</sup> and *Cajanus cajan*<sup>[12,13]</sup> have been investigated. Information on the gross and

developmental anatomy of different tissues such as root and hypocotyl of *Lablab purpureus* is lacking. Therefore, the present research work was undertaken to investigate the anatomical features of the hypocotyl of country bean at different stages of plant growth.

## MATERIALS AND METHODS

Mature seeds of country bean (*Lablab purpureus* (L.) Sweet) were collected from the Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh. The experiment was carried out in the BAU farm as well as in the Department of Crop Botany, BAU, Mymensingh, from August 2000 to March 2001. The seeds were sown in earthen pots. The earthen pots were filled up with thoroughly prepared soil of the plots. Some seedlings of the earthen pots were transplanted in the pits of the experimental plots. The earthen pots were kept exposed to the normal weather condition so that the plants of both earthen pots and plots got more or less similar weather conditions<sup>[14,15]</sup>. Some seeds were also placed on moist filter paper in petri dishes in the laboratory at room temperature of about 26-28°C. The petri dishes were kept in dark for about 24 h. The sprouting was considered as the zero hour of age of the plant<sup>[7,14-16]</sup>. For investigation, the plant samples were collected from the petri dishes, pots and plots and were fixed in Craff III<sup>[17]</sup> and in FAA<sup>[18]</sup> after making small pieces of about 5 mm in length<sup>[7]</sup>. The materials fixed in Craff III and FAA were dehydrated through the tertiary butyl alcohol (TBA) series<sup>[7,14,15,19]</sup>. The materials fixed in FAA were washed in running water for 2-4 h before dehydration. The hard materials (those fixed in FAA) were dehydrated through ethyl alcohol series while the soft and delicate materials (those fixed in Craff III) were dehydrated through tertiary butyl alcohol (TBA) series following the general principle of Johansen<sup>[18]</sup> and Sass<sup>[17]</sup>. The succulent materials were dehydrated gradually making more grades of alcohol to avoid severe shrinkage<sup>[14,15,19,20]</sup>. The dehydrated materials were then gradually infiltrated with heavy-duty paraffin oil and low melting point paraffin wax (49-51°C) for 2-3 days<sup>[7,19]</sup>. After infiltration the materials were embedded in high melting point paraffin wax (61-63°C). There was less shrinkage when the materials were infiltrated for a longer period<sup>[16,20]</sup>. Serial transverse sections of the wax embedded materials were obtained at 10 µ using a rotary microtome. The fresh and fixed materials were also sectioned by hand with ordinary razor blades. The hand sections were stained with safranin dissolved in 30% alcohol solution and temporary slides were made<sup>[18]</sup>. Finally, the sections were stained with safranin and fast green and mounted in

Canada balsam after proper dehydration with ethyl alcohol and clearing with xylene<sup>[18,19]</sup>. Olympus binocular compound microscope (Japan) has been used to investigate the anatomical sections.

## RESULTS AND DISCUSSION

**Epidermis:** The epidermis of the hypocotyl of country bean is single layered (Fig. 2, 3 and 9). The epidermis consists of small and large cells. Both the cells are radially elongated as seen in transverse section of the hypocotyl of 2 days old plant (Fig. 2). At the young hypocotyl the epidermal cells are thin walled. The epidermal circumference of the hypocotyl is more or less wavy (Fig. 1). The epidermal cells have been found to be regular in shape, size and arrangement as seen in transverse sections of the hypocotyl of 4 days old seedling (Fig. 3). With the age, the circumference of the epidermis becomes smooth. Similar result has been reported by Bari and Prodhan<sup>[14]</sup>, Islam<sup>[21]</sup>. As the plant ages, the cells become oval, round polygonal, ovate or obovate in shape as seen in transverse sections of the hypocotyl of 5-7 days old seedlings (Fig. 3, 5 and 7). 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 the epidermis of the hypocotyl of 2 days old plant have been found to be almost equally thin (Fig. 2). The walls gradually become thick along with the age. The thickening initiates in the abaxial wall and gradually it extends to the lateral walls and ultimately to the adaxial walls as seen in transverse sections of the hypocotyl of 4-9 days old seedlings. The cuticle appears over the abaxial wall of the epidermis of 4 days old seedlings (Fig. 4). Gradually it thickens. The epidermis bears multicellular hairs (Fig. 4 and 5). The epidermis becomes ruptured and broken here and there and disorganized in the later stage of growth. This is probably due to the stress of secondary growth and sharp increase in girth.

**Cortex:** There are 16-20 layers of cortical cells in the hypocotyl of country bean (Fig. 2, 3 and 6). The number of cortical layer is more in the basal part and less in the apical part. The number of cortical layer at the basal part gradually decreases along with the age. All the cells of the cortical region are parenchymatous in nature. The abaxial and adaxial cells of the cortex are smaller than that of the middle region (Fig. 2, 3, 6 and 11). However, most of the cells in the middle region are larger in size. Most of the cells are round, oval or polygonal, some are hexagonal or pentagonal while the others are irregular in shape (Fig. 2, 3, 5, 10 and 11). In the cortical

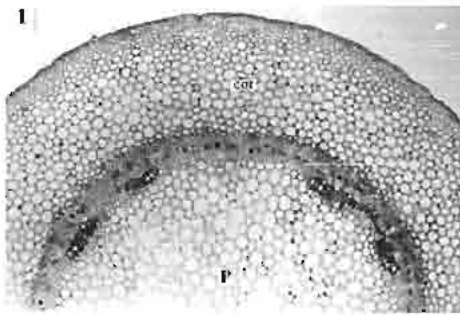


Fig. 1: T.S. of the basal part of the hypocotyl of a 2 days old plant showing epidermis (e), cortex (cor), vascular tissues and pith (p). Tannin cells are present in the primary phloem region. X 132

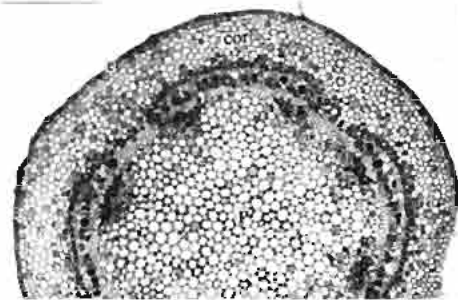


Fig. 4: T.S. of the apical part of the hypocotyl of a 5 days old plant showing epidermis (e), cortex (cor), vascular bundles and pith (p). X 132

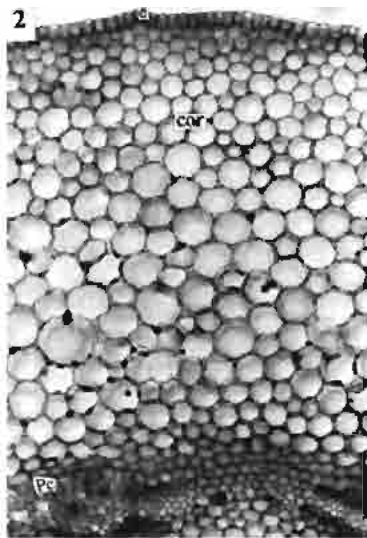


Fig. 2: Higher magnification of Fig. 1 showing epidermis (e), cortex (cor) and discontinuous pericycle (pc) X 260

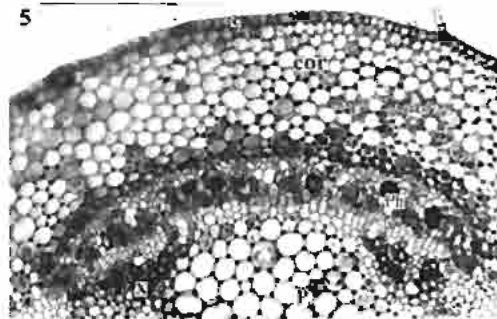


Fig. 5: Higher magnification of Fig. 4 showing epidermis (e) with cuticle and hairs, cortex (cor), xylem (x), phloem (ph) and pith (p). Tannin cells are present in the primary phloem region. X 260

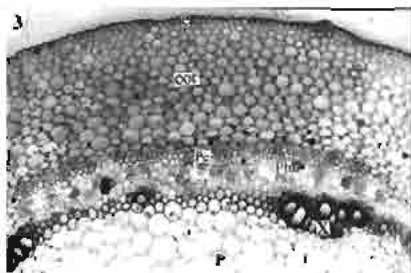


Fig. 3: T.S. of the basal part of the hypocotyl of a 4 days old plant showing epidermis (e) with cuticle, cortex (cor), phloem (ph), xylem (x), discontinuous pericycle and pith (p). X 240 X 260

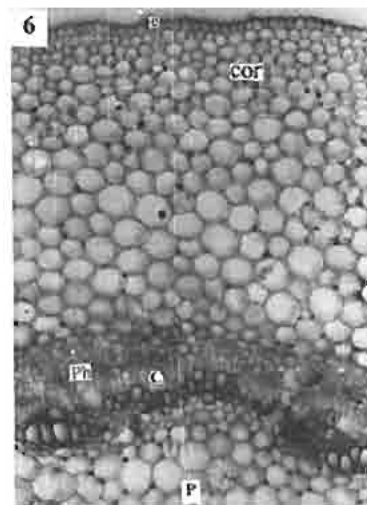


Fig. 6: T.S. of the basal part of the hypocotyl of a 5 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle, cambium (c), phloem (ph), xylem (x) and pith (p). X 280

parenchyma there are intercellular spaces. With the age, the intercellular spaces become prominent and the cortical cells become more or less tangentially flattened (Fig. 9, 10, 12, 17 and 18). The intercellular spaces also increase in number. The cortical cells of the hypocotyl seem to be smaller or similar to those of the root. Similar reports are available<sup>[14,21-23]</sup>. The cells of the outermost layer of the cortex are more or less similar to the cells of the epidermis specially at the middle or basal part of the younger hypocotyl (Fig. 3, 11 and 12). There is no or little intercellular spaces between this layer and epidermis.

The cells of the innermost layer of the cortex have not been found to contain starch. The cells of this layer are thick walled. The layer is darker or hyperchromatic in nature at the early stage of growth (Fig. 7, 8, 10 and 13). A thorough developmental study is required to disclose its similarity to the endodermis or to the starch sheath. The tannin cells have not been observed in the cortex of the hypocotyl of country bean during the present investigation. The tanniferous cells have been found in the cortex of the younger hypocotyl of lignosus bean<sup>[14]</sup>. The abaxial cells of the cortex are ruptured and broken here and there and disorganized in the later stage of growth. This is due to the stress of secondary growth and increase in girth.

**Primary vascular tissue:** The vascular tissues in the upper and middle parts of the hypocotyl of country bean are collateral in arrangement as seen in transverse sections (Fig. 4, 5, 11 and 14). In the basal part of the hypocotyl, the xylem tissues as well as xylem vessels are found to be more or less radially arranged (Fig. 1, 3, 6, 8 and 11). In *Brassica campestris*, the upper part of the hypocotyl is stem-like and the lower part is root-like in structure<sup>[23]</sup>. In lignosus bean, the hypocotyl is stem-like in structure<sup>[14]</sup>. The transition of vascular tissues occurs in the basal part of the hypocotyl. In the upper part of the hypocotyl, there are two types of vascular bundles, large and small. The vascular bundles are arranged in a ring. There are 1-2 small vascular bundles in between two large bundles. The large vascular bundle contains xylem and phloem but the small bundle may or may not contain both xylem and phloem. In the basal part of the hypocotyl, there are 4 strands of xylem alternating with phloem zones. Each strand of xylem is divided into two poles each of which is tangentially elongated (Fig. 1). Two poles of xylem are either apart or connected to each other.

The sclerenchymatous cells are present on the abaxial side of all large and small vascular bundles (Fig. 7, 9, 10, 12 and 13). It is pericycle. The pericycle is discontinuous in the hypocotyl of country bean (Fig. 3, 6, 12, 17 and 18). The discontinuous pericycle has been reported by Bari and Prodhan<sup>[14]</sup>, Islami<sup>[21]</sup>. Radially each group of

sclerenchyma consists of more or less 2-4 cells. Two adjacent groups are connected by one or two layers of sclerenchyma. Sometimes 1-2 vascular bundles, either large or small, contain a single band of sclerenchymatous cells on their abaxial sides (Fig. 15 and 17). The sclerenchymatous cells contain prominent secondary thickening and large lumen. A continuous ring of sclerenchymatous fibres constituting the pericycle has been reported for pigeonpea stem<sup>[12]</sup>. Such sclerenchymatous fibres have different origin in different plants<sup>[22,24,25]</sup>. Therefore, the origin of this sclerenchyma tissue of country bean needs a thorough investigation.

**Primary xylem:** In the middle and upper parts of the hypocotyl, the large vascular bundle contains xylem and phloem but the small bundle may or may not contain both xylem and phloem (Fig. 4, 7, 10 and 15). 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 (Fig. 5, 7, 10 and 14). In the vascular bundle most of the vessels are arranged in radial rows while the 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 (Fig. 10, 14, 16 and 17). The mature vessel contains thick secondary wall and large lumen. It is completely devoid of protoplasm. In the basal part of the hypocotyl, the xylem vessels are arranged in tangential rows in the younger plant (Fig. 3 and 6) while in the older plants they are arranged in both radial and tangential rows (Fig. 9, 11, 13 and 18). The xylem vessels are placed side by side in such a way that the individual vessel members are found to be radial in the younger hypocotyl. In a single strand, there are two poles of xylem arranged in tangential rows (Fig. 1).

The vessels are round, oval or polygonal in shape as seen in the transverse sections. In the upper part of the hypocotyl, there are 6-10 vessel members in the large vascular bundle and 2-5 vessel members in the small vascular bundle. Bari and Prodhan<sup>[14]</sup> have reported 1-4 vessel members in the small vascular bundle and 4-8 vessel members in the large vascular bundle of the hypocotyl of lignosus bean. 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 the hypocotyl till the activity of the cambium continues. No secondary growth has been observed in the basal part of the hypocotyl till it is 5-6 days old.

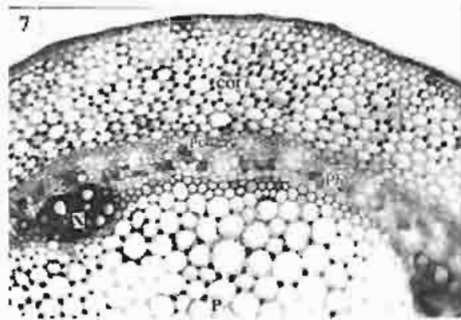


Fig. 7: T.S. of the middle part of the hypocotyl of a 7 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle (pc), cambium, phloem (ph), xylem (x) and pith (p). X 280

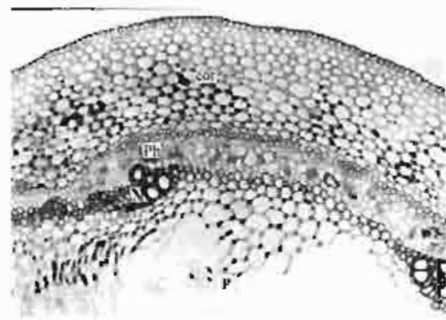


Fig. 10: T.S. of the middle part of the hypocotyl of a 10 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), phloem (ph), xylem (x) and pith (p)

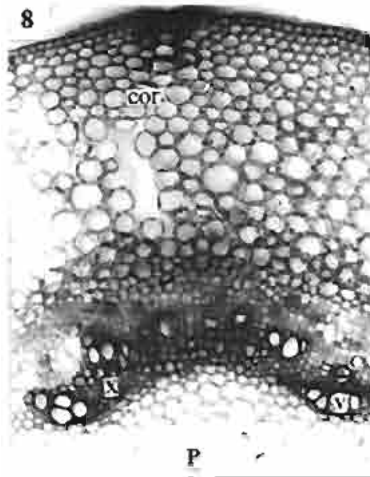


Fig. 8: T.S. of the basal part of the hypocotyl of a 7 days old plant showing epidermis (e) with cuticle, cortex (cor), cambium (c), phloem, xylem (x), xylem vessel (v) and pith (p). X 290

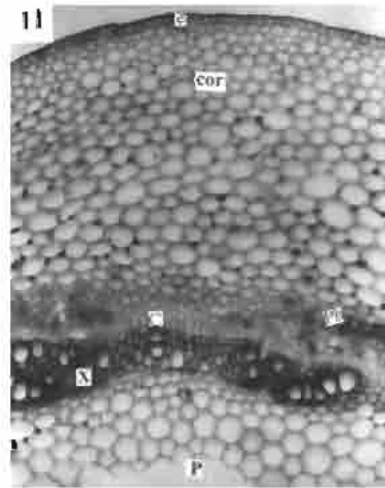


Fig. 11: T.S. of the basal part of the hypocotyl of a 10 days old plant showing epidermis (e) with cuticle, cortex (cor), cambium (c), phloem (ph), xylem (x) and pith (p). X 260

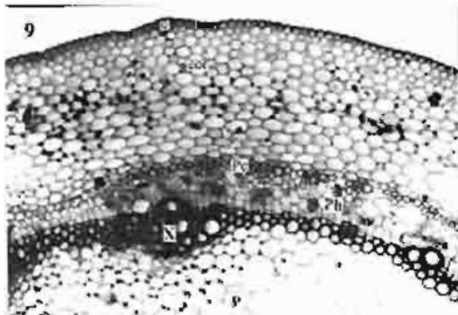


Fig. 9: T.S. of the basal part of the hypocotyl of a 9 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x) and pith (p). X 280

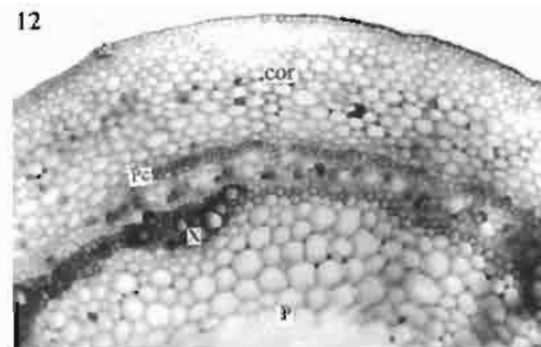


Fig. 12: T.S. of the apical part of the hypocotyl of a 12 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium, phloem (ph), xylem (x) and pith (p). X 260

**Primary phloem:** In the large vascular bundle there are a number of sieve elements and phloem parenchyma in the phloem zone (Fig. 5). Like primary xylem they mature rapidly. In the small vascular bundle there may be only one mature or immature sieve element. In the upper and middle parts of the hypocotyl, there are several poles of sieve elements abaxial to the primary xylem (Fig. 5 and 7). The number of phloem poles increases along with the age. There are several phloem poles in between two vascular bundles outside the xylem region. The phloem poles are arranged in a ring outside the xylem area (Fig. 4 and 5). In the basal part of the hypocotyl, there are several poles of phloem outside the primary xylem (Fig. 3, 8 and 9). There are also many poles of phloem in between two xylem strands (Fig. 9 and 18). The sieve elements are separated from each other by phloem parenchyma. Each pole consists of 2-5 sieve tube elements. In many cases sieve tubes have been found to accompany their companion cells. The sieve elements have also found to be scattered as seen in transverse sections. They are well apart, some ones are closer and some are apart. The primary sieve elements are continued to form in the hypocotyl till the plants are 5-6 days old after which secondary sieve elements begin to form.

Lots of tanniniferous cells with or without content have been found in the primary phloem region adaxial to the sclerenchymatous band in the hypocotyl (Fig. 1, 3, 7, 13 and 18). The tanniniferous cells have been observed in the younger hypocotyl but not in the older ones of the lignosus bean<sup>[14]</sup>. However, tannin cells have been found in the primary phloem region of the hypocotyl of country bean during the present investigation.

**Cambium:** The cambium has been found to initiate in the basal part of the hypocotyl of 4 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 (Fig. 3). 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. Metcalfe and Chalk<sup>[26]</sup> 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 4 days old seedling no activity has been observed till the plant is 5-7 days old (Fig. 6, 7 and 8). The

cambium forms a continuous ring in the basal part of the hypocotyl of 7 days old seedling excepting a few places of interfascicular regions where cortical tissue has been found to be continuous with the pith (Fig. 8). The cambial ring becomes complete later on (Fig. 9). A complete ring has been found in the basal part of the hypocotyl of 9 days old seedling (Fig. 9). In lignosus bean, a complete cambial ring has also been observed in the hypocotyl of 7 days old seedlings<sup>[14]</sup>. A continuous cambial ring has also been observed in the middle and upper part of the hypocotyl of 10-14 days old seedling. The cambial zone consists of several layers of tangentially flattened cells. It has been found to remain active up to the 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 5-6 days old seedling (Fig. 6). Gradually it extends towards the upper part of the hypocotyl as seen in transverse sections. The cambium appears and becomes active in the fascicular region earlier than the interfascicular region (Fig. 7, 9, 12 and 14). 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 the secondary tissues. According to Metcalfe and Chalk<sup>[26]</sup> the cambium is at first confined to the 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 of secondary growth the secondary tissue is more in the fascicular region than that of the interfascicular region (Fig. 7, 9, 10 and 13). 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. 8). The secondary xylem vessels are also observed at the middle and upper parts of the hypocotyl of 10-15 days old seedling (Fig. 10, 14 and 17). No secondary xylem has been found to form in the interfascicular region where no cambium has yet been formed as seen in transverse sections. In lignosus bean, no secondary xylem has been found in the upper part of the hypocotyl of 9 days old seedling<sup>[14]</sup>. 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,

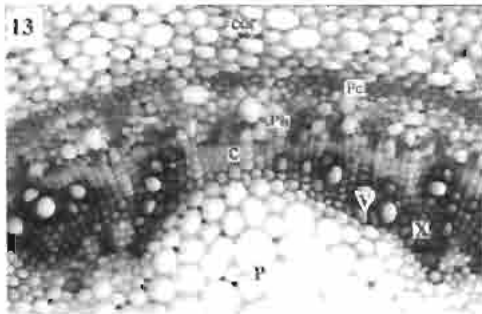


Fig. 13: T.S. of the basal part of the hypocotyl of a 12 days old plant showing cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260

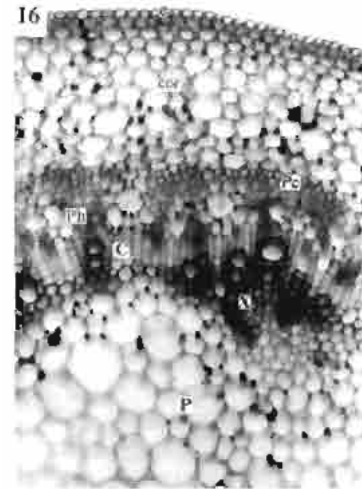


Fig. 16: T.S. of the middle part of the hypocotyl of a 14 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (sp), xylem (x) and pith (p). X 290 X 260

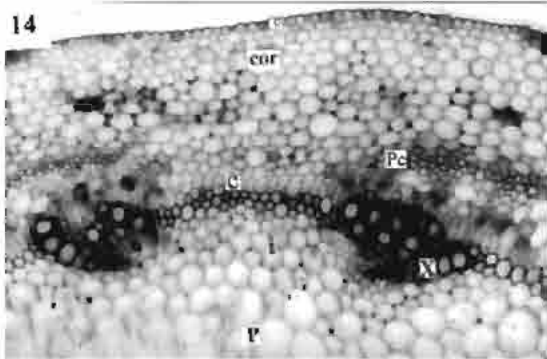


Fig. 14: T.S. of the middle part of the hypocotyl of a 13 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (sp), xylem (x) and pith (p). X 290

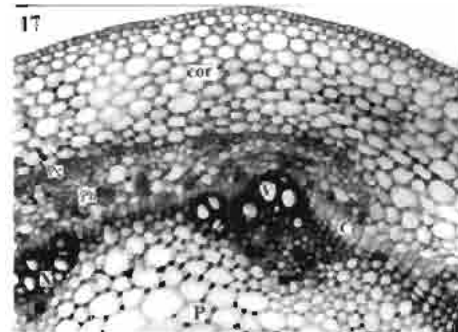


Fig. 17: T.S. of the apical part of the hypocotyl of a 15 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 280

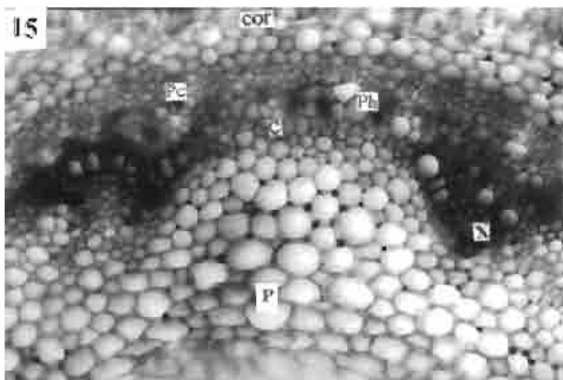


Fig. 15: T.S. of the apical part of the hypocotyl of a 14 days old plant showing cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x) and pith (p). X 290

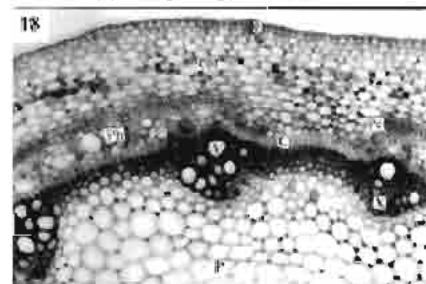


Fig. 18: T.S. of the basal part of the hypocotyl of a 15 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260



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<sup>[21]</sup>. Similar result has been reported in the hypocotyl of lignosus bean by Bari and Prodhan<sup>[14]</sup>. Lots of secondary xylem are found in the hypocotyl of mature plant (Fig. 19, 20 and 21). 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 the present investigation. Some attention has, however, been given on the vessel members, axial and ray parenchyma and fibres. Some of the secondary xylem vessels are arranged more or less radially and others are scattered as seen in transverse sections. Most of the vessels are found in individual groups. Some vessels are multiple, some are solitary while a few are paired (Fig. 19, 20 and 21). Small vessels are radially arranged. The spaces between the secondary xylem vessels are filled up with other elements of secondary xylem like ray, axial parenchyma and fibres.

The axial xylem parenchymatous cells have been found in between and around the vessel members. They are thick walled. The ray cells are small. They are mostly multiseriate, some are biseriate and a few are uniseriate and become gradually thickened. The ray cells are less thickened than the axial xylem parenchyma of secondary origin. The fibre cells are found in either groups or scattered. They are present in and around the vessel members. The fibres are highly lignified, thick walled with small lumen. They are mostly hexagonal or pentagonal in shape. Similar result has been reported for lignosus bean<sup>[14]</sup> and *Brassica campestris*<sup>[23]</sup>. With growth and maturity, secondary xylem pushes the primary xylem into the centre. 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 5-6 days old seedling (Fig. 6). 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 and axial phloem parenchyma have been found to form in the hypocotyl of 7-12 days old plants (Fig. 8, 9, 11 and 13). 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. So by size one cannot easily recognize primary and secondary sieve elements at this stage of growth.

Some phloem poles are present even where there is no corresponding xylem (Fig. 1, 4 and 5). There is a group of sieve elements in large vascular bundle as seen in 5-9 days old hypocotyl (Fig. 5, 7, 8 and 11). There are 3-5 sieve tube elements in the large vascular bundle and 1-3 sieve tube elements in the small vascular bundle. There are 1-5 sieve tube elements in the vascular bundle of lignosus bean<sup>[14]</sup>. The phloem zone is narrow. Radially it consists of 3-5 layers of cells as seen in the hypocotyl of 5-9 days old plant (Fig. 5, 7 and 9). The thickness increases in the hypocotyl of mature plant (Fig. 21 and 22).

Both axial and ray parenchyma of secondary origin are also limited in number in young plant while numerous in mature plant. The ray cells are large as seen in transverse sections. Some hyperchromatic phloem parenchyma have also been observed in the phloem region. Hyperchromatic phloem parenchyma has been found in the phloem region of the hypocotyl of lignosus bean<sup>[14]</sup>. The fibre cells have been observed in the secondary phloem during the present investigation. Almost all of the secondary phloem fibres are found in groups while a few are scattered. The secondary phloem fibre groups are radially arranged in such a way that the fibre groups make a pyramid like structure (Fig. 21 and 22). Abaxial to the pyramid there are groups of primary phloem fibres. The primary phloem fibres are thick walled with small lumen. Tannin cells have been found in the pyramid like structure. Tannin cells without content have also been observed in the phloem region. The secondary phloem continues to form and the sieve elements remain active till the senescence of the plant.

**Pith:** The pith is prominent in the hypocotyl of country bean (Fig. 1 and 4). 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. 1, 4, 7, 15 and 18). The pith increases in size due to the 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



Fig. 19: T.S. of the hypocotyl of a mature plant showing cambium (c), secondary xylem (sx), secondary phloem (sp), secondary xylem vessels (v), fibres (F), ray and axial parenchyma. X 290

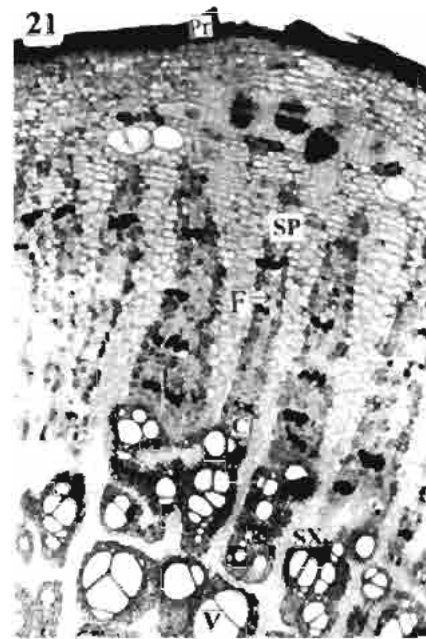


Fig. 21: T.S. of the hypocotyl of a mature plant showing periderm (Pr) with lenticel, cambium (c), secondary xylem (sx), secondary phloem (sp), secondary xylem vessel (v), phloem fibres (F). Tannin cells are present in the phloem region. X 290

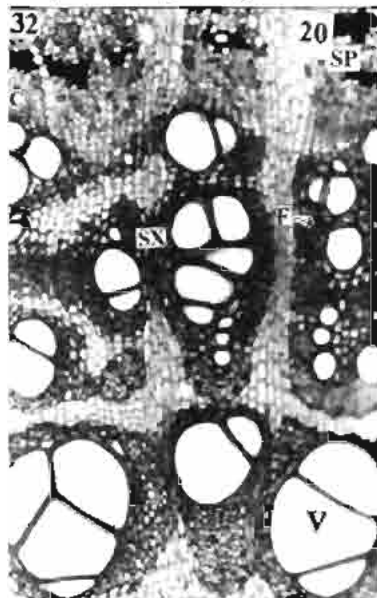


Fig. 20: T.S. of the hypocotyl of a mature plant showing secondary phloem (sp), cambium (c), secondary xylem (sx), secondary xylem vessel (v), xylem fibres (F), ray and axial parenchyma. Phloem contains tannin cells. X 280



Fig. 22: T.S. of the hypocotyl of a mature plant secondary phloem (sp), phloem fibres (F). Most of the fibre cells are arranged in groups. Phloem region contains tannin cells. X 320

in size<sup>[21]</sup>. Similar findings have been reported for *Brassica campestris*<sup>[23]</sup> and lignosus bean<sup>[14]</sup>. 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 centre of the pith of older hypocotyl. Most of the cells in the pith are larger in size. Due to the stress of axial growth of the hypocotyl and the addition of secondary xylem towards the centre, the peripheral pith cells lose their intercellular spaces and become narrow but the pith cells at the centre remain more or less unaffected. In the mature plant, the pith of the hypocotyl becomes small due to the secondary growth.

**Periderm:** The periderm forms in the hypocotyl of country bean (Fig. 21). 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. 21). In Papilionaceae, similar result has been reported by Cutter<sup>[22]</sup> and Esau<sup>[24]</sup>. The number of cork cells in a radial row depends on the age and size of the plant or plant parts<sup>[24,27]</sup>. The initiation of phellogen and the development and morphology of different components of periderm have not been studied thoroughly during the present investigation. The phellogen produces 3-5 layers of cork cells abaxially and 1-3 layers of phelloderm adaxially. In the hypocotyl of lignosus bean, the phellogen produces 4-6 layers of cork cells and 2-3 layers of phelloderm<sup>[14]</sup>. 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 periderm of country bean bears lenticel (Fig. 21). The cells of phelloderm resemble cortical cells but they are smaller in size. The development of periderm in the hypocotyl of country bean needs further investigation.

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