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Anatomy of Lignosus Bean (*Dipogon lignosus*) I: Root

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Abstract: Anatomical investigation has been made on the root of lignosus bean (*Dipogon lignosus* (L.) Verdc.) at different stages of growth following the standard paraffin method of micro technique. The primary root is tetrarch with 4 strands each of xylem and phloem that alternate with one another. Metaxylem and metaphloem follow protoxylem and protophloem respectively in their courses of differentiation. The four opposite strands of primary xylem meet at the center. Subsequently, metaxylem forms near the center on either side of the xylem strands. The epidermis is single layered with large number of root hair and glandular trichomes. There are 6-12 layers of cortical cells beneath the epidermis. The adaxial layers (radially 4-6 cells) of the cortex around the stele are hyperchromatic in nature. The hyperchromaticity is higher in the cells abaxial to xylem poles. The cambium appears in the upper part of the root and extends towards the root apex. The sclerenchymatous band abaxial to phloem is discontinuous. The vessels are more or less round, oval or polygonal in shape with prominent secondary thickening. The phellogen appears in the cortex, 3-4 layers beneath the epidermis. It produces 3-5 layers of cork cells abaxially and 2-3 layers of phelloderm adaxially.

Key words: Lignosus bean, *Dipogon lignosus*, anatomy, root.

Introduction

Lignosus bean [*Dipogon lignosus* (L.) Verdc. (Syn. *Dolichos lignosus* L., *D. benthamii* Meisn., *D. gibbosum* Thumb., *Verdcourtia lignosus* (L.) Wilczek), 2n = 20)] belongs to sub-family Papilionaceae under the family Leguminosae. It is a wild species of hyacinth bean [*Lablab purpureus* (L.) Sweet (Syn. *Dolichos lablab* L., *D. purpureus* L., *Lablab niger* Medik), 2n = 22, 24] (Verdcourt, 1970; Zeven and De Wet, 1982). Hyacinth bean is also known as Indian bean, lablab bean, seim bean or country bean. There are about 50 species of hyacinth bean distributed throughout the world (Bailey, 1949). This self pollinated crop is probably originated in India and can be grown from sea-level to 7,000 ft in Asia. It is also cultivated in Africa, S. America and Australia where it has run wild (Verdcourt, 1970). The lignosus bean is grown as a field crop in Asia, mainly for the ripe seeds and fodder (Purseglove, 1974). The dry seeds of lignosus bean contain about 22.3% protein (Bari, 2000).

External morphological characters of different types of bean plants are known to some extent (Purseglove, 1974; Verdcourt, 1970) but the information on anatomical features of these plants is very scanty. Available literature shows that some anatomical works on the root so far have been done with papilionaceous plants, but no work has been carried out with bean plants. Some developmental works have been carried out with the root of *Sesbania rostrata*, *S. sesban* and *S. formosa* (Hossain, 1997; Sarker, 1996). The development of different tissues such as root apices of *Cicer arietinum* and *Vicia faba* (Eames and MacDaniels, 1953), root cortex of *Robinia* (Feher, 1924), vascular tissues of the root of Papilionaceae (Esau, 1977; Pandey, 2001) and periderm of the root (Esau, 1965; Pandey, 2001) has been investigated.

However, information on the gross and developmental anatomy of different tissues of the root of lignosus bean is lacking. The objective of the present work is to investigate the anatomical features of the root of lignosus bean (*Dipogon lignosus* (L.) Verdc.) at different stages of growth.

Materials and Methods

Mature seeds of lignosus bean (*Dipogon lignosus* (L.) Verdc.) 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 with thoroughly prepared soil of the plots. The seedlings of polybags were transplanted in the pits of 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. Some seeds were also placed on moist filter paper in large petri dishes in the laboratory at room temperature of about 26-28 ^{o}C. The petri dishes were kept in dark for about 24 hours. The sprouting was considered as the zero hour of age of the plant.

Roots of 1, 2, 4, 5, 7 and 9 days old seedlings were collected from the petri dishes and polybags, and were fixed separately in Craf III (Sass, 1958) after making small pieces of about 5 mm in length. The roots of 5, 7, 8, 9 and 10 days old seedlings were collected from both polybags and pits of the plots. The roots of 15, 20, 50, 180 and 210 days old (matured) plants were also collected from the pits of the plots. These were fixed in FAA (Johansen, 1940) after making pieces of about 5 mm in length. The materials fixed in Craf III and FAA were dehydrated through the tertiary butyl alcohol (TBA) series on the general principle of Johansen (1940) and Sass (1958). The materials fixed in FAA were washed in running water for 2-3 hours before dehydration. The materials fixed in Craf III were very succulent. They were dehydrated gradually making more grades of alcohol to avoid severe shrinkage (Ali *et al.*, 1999; Haque and Engleman, 1978; Haque and Prodhan, 1991; Prodhan and Haque, 1986).

The dehydrated materials were gradually infiltrated with paraffin oil and low-melting-point paraffin wax (51 ^{o}C) for 1-3 days. The succulent materials were dehydrated for a longer period. Finally the materials were embedded in high-melting-point paraffin wax (61 ^{o}C). Repeated trial showed that there was less shrinkage when the materials were infiltrated for a longer period (Haque and Prodhan, 1987; Prodhan and Haque, 1986; Sarwar and Prodhan, 2000). 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 (Johansen, 1940). Free hand sections were also made from fresh and fixed roots (Prodhan and Haque, 1986). Olympus binocular compound microscope (Japan) has been used to investigate the anatomical sections.

Results and Discussion

Epidermis: The epidermis of the lignosus bean (*Dipogon lignosus*) is single layered. The epidermal cells are irregularly arranged as seen in the transverse sections of the tip of the root/radicle of two days old seedling (Fig. 1). Some epidermal cells are small and some are large. Most of the cells towards the distal part of the root give rise to root hair and glandular trichomes. The root hair are unicellular and the glandular

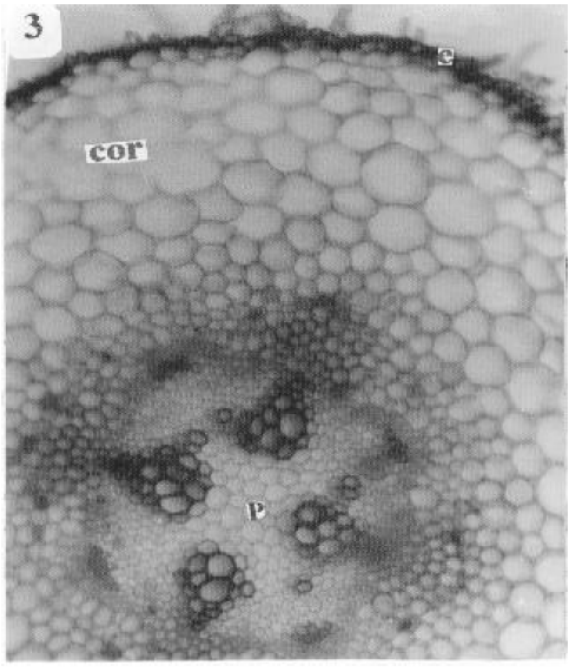

Fig. 1: T.S. through the apical part of the root (about 0.5 mm away from the tip) of a 2 days old plant, showing epidermis (e) with root hair, cortex (cor), 4 poles of xylem and 4 poles of phloem. Each pole of xylem contains protoxylem and metaxylem vessels and each pole of phloem contains mature and immature sieve elements. Around the stele there are lots of hyperchromatic parenchyma cells. Hyperchromaticity is higher on the abaxial side of the xylem poles. X 180.

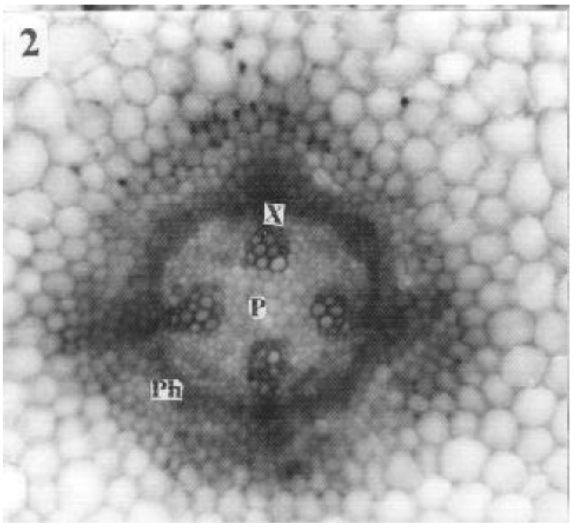

Fig. 2: Higher magnification of the central region of Fig. 1 showing xylem pole (x), phloem pole (ph) and pith (p). Xylem pole shows protoxylem vessels, mature and maturing metaxylem vessels. Phloem pole shows mature and immature sieve tube elements. Around the stele there are lots of hyperchromatic parenchyma cells. Hyperchromaticity is higher on the abaxial side of the xylem poles. X 290.

Fig. 3: T.S. of the basal part of the root of a 4 days old plant showing epidermis (e) with root hair, cortex (cor), pith (p), 4 poles of xylem and 4 poles of phloem. Each pole of xylem contains protoxylem, and mature and maturing metaxylem vessels. Each pole of phloem contains mature and immature or maturing sieve elements. Around the stele there are lots of hyperchromatic parenchyma cells. Hyperchromaticity is higher on the abaxial side of the xylem poles. X 360.

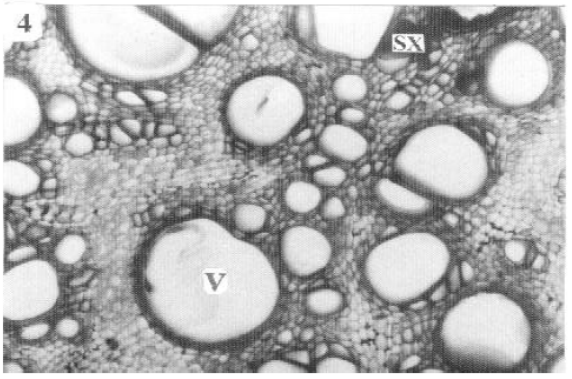

Fig. 4: T.S. of the root of a 180 days old (mature) plant showing secondary xylem (sx) and secondary xylem vessels (v). Vessels are large and small. There are lots of axial and ray parenchyma and a few fibre cells. X 320.

trichomes are multicellular. The outer wall of the epidermis of two days old root/radicle seems to be slightly thickened probably due to the formation of thin cuticle. The cells at this stage are irregular, oval, or slightly rectangular in shape as seen in fresh, fixed and dehydrated materials.

As the root elongates the epidermal cells in the upper region of the root gradually become regular as seen in transverse sections of the root of 4 days old seedlings. The epidermal cells at this stage of growth become somewhat tangentially flattened. The outer walls of which are thicker than the adaxial and lateral walls. The large and small cells have been found in the epidermis of the root of *Brassica campestris* (Prodhan and Haque, 1986). The larger cells have been found to be unevenly distributed in the smaller cells of the epidermis. There may be one or more large cells at a place (Prodhan and Haque, 1986). During present investigation

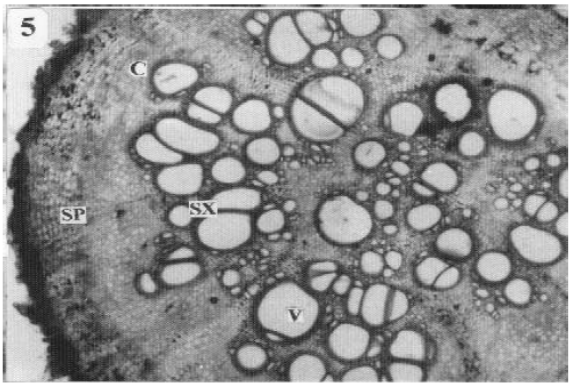

Fig. 5: T.S. of the root of a 210 days old (mature) plant showing cambium (c), secondary phloem (sp), secondary xylem (sx) with large and small secondary xylem vessels (v) and periderm (Pr). There are lots of axial and ray parenchyma and a few fibre cells. X 132.

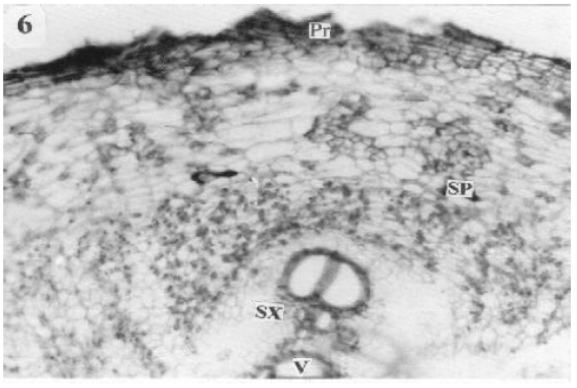

Fig. 6: T.S. of the root of a 210 days old (mature) plant showing secondary xylem (sx), xylem vessels (v), secondary phloem (sp) and periderm (Pr). Secondary phloem shows several groups of phloem fibres and parenchyma cells. Periderm shows cork cells, phellogen and phelloderm. X 320.

large and small cells have been found in the epidermis of the root of lignosus bean but no sequence has been observed in their arrangement. The size of the cells has been considered by diameter and not by length (Prodhan and Haque, 1986). So far the composition of epidermis of the root of lignosus bean is concerned the present finding agrees with the work of Prodhan and Haque (1986).

Along with the age, the tangentially flattened cells gradually become more or less radially elongated. The abaxial walls of epidermis become more thickened. The epidermal cells have been found to be more regular in shape. The regularity has been observed in both large and small cells as seen in the transverse sections of the root of 4 days old seedling (Fig. 3). Along with the age the epidermal cells, both small and large, become smaller in size as seen in the transverse sections. The epidermis becomes disrupted here and there as seen in transverse sections of older roots (Fig. 6). This is probably due to the stress of secondary growth and sharp increase in girth (Esau, 1977; Pandey, 2001).

Cortex: There are 6-12 layers of cortical cells in the root of lignosus bean (*Dipogon lignosus*). Hossain (1997) has reported 7-10 layers in the root of *Sesbania formosa*. The lower part of the root of lignosus bean contains comparatively less number of cortical layers while the upper part contains more. In the root of 2 days old seedling, the cortical tissue is radially 6-8 layered in the lower part and 8-12 layered in the upper part (Fig. 1, 3). The young cortical cells contain small intercellular spaces. As the root/radicle elongates the diameter of the root decreases and the size of the cortical cells becomes smaller (Prodhan and Haque, 1986) but the number of cortical layers remain more or less same as seen in transverse sections. At later stages of primary growth, the number of layers of cortical tissue slightly increases particularly at the upper part of the root. The number of intercellular spaces increases along with the age. All the cells of the cortex become somewhat round, oval or polygonal in shape as seen in 2 days old root. The cells of the middle layers are larger in size. The cells are thin walled with conspicuous intercellular spaces. The abaxial and adaxial cells of the cortex are smaller in size. One row of the cells gradually organizes around the stele to form endodermis. The adaxial layers (radially 4-6 cells) of the cortex around the stele are hyperchromatic in nature. The hyperchromaticity is higher in the cells abaxial to xylem poles. No tanniniferous cells have been observed in the cortex of root of lignosus bean during the present investigation. However, tanniniferous cells in the cortex of the root of Papilionaceous plants like *Sesbania rostrata* and *Sesbania sesban* have been reported by Sarker (1996). Lots of tannin cells are present in the root of *Robinia* (Feher, 1924). As the diameter of root increases the cortical cells become tangentially flattened. The abaxial cells become ruptured and broken here and there and disorganized as seen in the older roots.

Primary vascular tissue: The primary root shows a tetrarch proto stele (Fig. 1, 2, 3). Four poles each of xylem and phloem appear in the hypocotyl-root axis of one day old seedling. One strand of xylem alternates with one strand of phloem. Similar results have been reported for *Cicer arietinum* and *Vicia faba* (Eames and MacDaniels, 1953). The poles of both xylem and phloem extend towards the distal part as the radicle extends. Serial cross sections reveal that the radicle contains mature tracheary and sieve elements all the way from the hypocotyl to the radicle except the radicle tip of about 0.5 mm. Adaxial to protoxylem, metaxylem begins to differentiate rapidly. Gradually the centre is filled with big metaxylem vessels (Prodhan and Haque, 1986). The cambium initiates in the root of 3 days old seedling and begins to cut off secondary tissues within 3-4 days.

Primary xylem: There are 4 poles of xylem in the root/radicle of 2 days old seedlings. In the lower part of the root each pole of xylem contains one immature or developing protoxylem vessel. In the middle part it contains one mature protoxylem vessel and one or more metaxylem vessels while in the upper part it contains one protoxylem vessel and more metaxylem vessels (Fig. 1). The metaxylem in the upper part consists of both mature and immature vessels. The mature vessel members are completely devoid of protoplasm and they contain conspicuous secondary thickening in their cell walls (Prodhan and Haque, 1986). The immature vessel member shows shrunken protoplasm and some secondary thickening in the wall. The 4 xylem poles are well aparted from each other. There are large thin walled parenchymatous cells at the centre of the root, which is known as pith (Fig. 1, 3). The xylem poles are evident all the way of the hypocotyl-root axis except the tip at this stage of growth.

The number of xylem vessels gradually increases in each pole. In the middle part of the root of 4 days old seedlings each pole

generally contains 14-16 vessel members of which 7-10 are mature (Fig. 3). The vessels are more or less round, hexagonal or polygonal in shape with prominent secondary thickening (Prodhan and Haque, 1986). The bigger vessels are adaxial while the smaller ones are abaxial. In lower part of the root, smaller vessels are in the same radius as those of the bigger vessels but in the upper part, they are not in the same radius. Subsequently more elements differentiate near these vessels on either side of the axis of 4 poles of protoxylem. The subsequently formed vessels are big with large lumen and thick secondary walls. No secondary growth has been observed in the primary root till it is 5 days old.

Primary phloem: There are 4 poles of phloem in the root of 2 days old seedlings (Fig. 3). Immature sieve elements are present in the hypocotyl-root axis. Each pole consists of protophloem and metaphloem sieve elements (Prodhan and Haque, 1986). In the lower part, each pole contains one immature or differentiating sieve element. In the middle part, it contains one mature protophloem and one or more metaphloem sieve elements, while in the upper part, it contains one mature protophloem and more metaphloem sieve elements. In the upper part the metaphloem consists of both mature and immature sieve elements. The wall of the protophloem sieve element stains more deeply than those of the surrounding cells. Similar phenomenon has been reported for *Corchorus* species (Haque and Isa, 1977). The root apex of about 0.5 mm in length does not contain any mature or differentiating sieve elements. The sieve element is the protophloem sieve tube member and accompanied by hyperchromatic phloem parenchyma without any companion cell. In the root of *Phaseolus mungo*, the protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma and it is detected in the hypocotyl-root axis (Haque and Engleman, 1978; Haque and Isa, 1977). In root, the protophloem sieve element normally lacks companion cells (Esau, 1965). The 4 poles of phloem are well aparted from each other as compared to xylem poles (Fig. 2).

The number of sieve elements in each pole increases along with the age. Several sieve elements have been found in each pole of phloem as seen in 4 days old roots (Fig. 3). Some of the metaphloem sieve tubes contain companion cells in the upper part of the root (Prodhan and Haque, 1986). The subsequently formed sieve tubes are slightly bigger. The primary sieve elements are continued to form in the roots till the plants are 5 days old after which secondary sieve elements begin to form.

Cambium: The cambium has been found to form in the upper part of root of 3 days old seedling. Gradually it extends towards the tip. Within 3-4 days of initiation, the cambium forms a ring (cambial ring) and begins to form secondary tissues. The cambium is active in all places of the ring. The cambium produces secondary xylem adaxially and secondary phloem abaxially. The cambium has been found to be more active in its adaxial side (Prodhan and Haque, 1986). The cambial zone consists of several layers of tangentially flattened compact cells. The initials and derivatives have not been studied during the present investigation. The derivatives of the cambium showed more vacuolation on its adaxial side. The cambium has been found to remain active till the senescence of the plant.

Secondary xylem: The secondary xylem begins to form in the upper part of root of 6-7 days old plant. The cambium gives rise to different elements of secondary xylem and ray cells on its adaxial side. The differentiation of different cells derived from cambial initials has not been studied during the present investigation. However, some attention has been given on the differentiation of vessel members from the cambium. The mature secondary vessel members have been found in the root of 7-9 days old plant. They are fully devoid of protoplasm at this stage having polygonal, round or oval in shape with prominent secondary thickening (Prodhan and Haque, 1986). The secondary xylem continues to form with the age. Lots of xylem vessels have been found in the mature root (Fig. 4, 5). The vessel members are large, medium and small in size. Most of the vessels are scattered and the others are arranged in pairs. In *Sesbania sesban*, most of the vessels are found in pairs and arranged radially throughout the xylem area (Sarker, 1996). The spaces between the vessels are filled up mostly with parenchyma and fibre cells. The fibre cells are thick walled with small lumen. The secondary xylem parenchymatous cells are thick walled. The ray cells are arranged radially. The ray parenchyma is also thickened to some extent. There are axial parenchyma around vessels. The axial parenchyma cells are also thick walled. The structures of secondary xylem in root of lignosus bean (*Dipogon lignosus*) are similar to those of many slightly woody herbaceous dicotyledonous plants (Esau, 1977; Prodhan and Haque, 1986). In the upper part of root, the secondary xylem pushes the primary xylem towards the centre. The elements of primary xylem both protoxylem and metaxylem remain intact near the centre (Fig. 5).

Secondary phloem: The secondary phloem begins to from in the upper part of the root of 6-7 days old plant. The secondary sieve elements have been found to form as an activity of the cambium. The sieve elements of the root of 6-7 days old plant are mostly primary in origin. Several strands/poles of secondary sieve elements have been found in 6-7 days old root. The sieve elements are at various stages of development at this age. In a single strand there may be one or more sieve elements (Prodhan and Haque, 1986). It is difficult to distinguish secondary elements from the primary by the position because the secondary elements might have appeared in places where there were no primary elements. The diameters of metasieve tube members and the secondary sieve elements have been found to be more or less same as seen in transverse sections. Therefore, one cannot distinguish primary and secondary sieve elements by size. The phloem zone is narrow consisting of 5-6 layers of cells as seen in transverse sections of the root of 6-7 days old plant. The number of sieve elements slightly increases with the age of plant (Fig. 5).

Periderm: The periderm normally forms in the root of lignosus bean. The epidermis ruptures here and there and the cells gradually disorganize (Pandey, 2001). After a partial or total disintegration of the epidermis the phellogen appears (Prodhan and Haque, 1986). It appears in the cortex, 3-4 layers beneath the epidermis (Fig. 6). The periderm normally originates in the root from a deep layer (Easu, 1965). The phellogen produces 3-5 layers of cork cells abaxially and 2-3 layers of phelloderm adaxially. The cork cells are apparently devoid of protoplasm and are thick walled (Pandey, 2001). They are tangentially flattened and brick shaped in appearance as seen in transverse sections (Fig. 6).

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