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Root Anatomy of Country Bean

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Abstract: Anatomical investigation has been made on the root of country bean (*Lablab purpureus* (L.) Sweet) at different stages of growth following the standard paraffin method of microtechnique. The root is tetrarch with 4 strands of xylem and 4 strands of phloem. One strand of xylem alternates with one strand of phloem. The four opposite strands of the primary xylem meet at the centre. Ultimately the centre is filled up with big metaxylem vessels. Most of the vessels in the mature root are solitary while the others are paired or multiple. The epidermis is single layered with root hairs and glandular trichomes. The epidermis is ruptured here and there and the epidermal cells are disorganized due to the stress of secondary growth. Soon after the disorganization of the epidermal cells the phellogen appears in the cortex. The cortex resembles a typical dicotyledonous plant excepting the endodermis, which is poorly developed. The cambium appears in the basal part of 4 days old root. In mature root, the fibre cells are arranged in groups. The fibre groups are radially arranged in such a way that the structure seems to be a pyramid. Adaxial to the phloem region, tanniniferous cells have been found. The protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma without any companion cell. The well-developed periderm has been found in the root of country bean.

Key words: Anatomy, country bean, *Lablab purpureus*, root

INTRODUCTION

Country bean (*Lablab purpureus* L.) Sweet, Syn. *Dolichos lablab* L., *D. purpureus* L., *Lablab niger* Medik) 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 (Verdcourt, 1970; Zeven and De Wet, 1982). 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-lived creeping perennial but cultivated 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 and 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 (Deka and Sarker, 1990; Newaz, 1992).

The anatomical research works of this plant have not been investigated thoroughly. Some sporadic works have been carried out with country bean plant at home and abroad. The development and structure of different tissues of *Dipogon lignosus* (Bari and Prodhan, 2001a, b; Prodhan and Bari, 2001), *Sesbania rostrata* (Prodhan and Sarker, 2002), *S. sesban* (Sarker and Prodhan, 2001), *Cajanus cajan* (Bisen and Sheldrake, 1981) and *Lablab purpureus* (Islam *et al*., 2003, 2005) have been investigated to some extent. Information on the gross and developmental anatomy of root of

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Lablab purpureus is very limited. Therefore, the present research work has been undertaken to investigate the anatomical features of the root 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, 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 (Islam *et al*., 2003, 2005; Prodhan and Bari, 2001). 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 0 h of age of the plant (Bari and Prodhan, 2001a, b; Islam *et al*., 2003, 2005; Prodhan and Bari, 2001). For investigation, the plant samples were collected from the petri dishes, pots and plot and were fixed in Craf III (Sass, 1958) and in FAA (Johansen, 1940) after making small pieces of about 5 mm in length. The materials fixed in Craf III and FAA were dehydrated through the tertiary butyl alcohol (TBA) series (Bari and Prodhan, 2001a, b; Haq and Prodhan, 1987; Islam *et al*., 2003, 2005). 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 Craf III) were dehydrated through tertiary butyl alcohol (TBA) series following the general principle of Johansen (1940) and Sass (1958). The succulent materials were dehydrated gradually making more grades of alcohol to avoid severe shrinkage (Haque and Prodhan, 1987; Islam *et al*., 2003, 2005; Prodhan and Bari, 2001; Prodhan and Haque, 1986). 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 (Haque and Prodhan, 1987). 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 (Islam *et al*., 2003, 2005; Prodhan and Bari, 2001; Prodhan and Haque, 1986). 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 (Johansen, 1940). 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 (Haque and Prodhan, 1987; Johansen, 1940). Olympus binocular compound microscope (Japan) has been used to investigate the anatomical sections.

RESULTS AND DISCUSSION

Epidermis

The epidermis is single layered. The epidermal cells are irregularly arranged in the basal part of 2 days old root (Fig. 3). Some epidermal cells are small and some are large, but no sequence in their arrangement. Most of the cells towards the distal part of the root give rise to root hairs and glandular trichomes (Fig. 3). Similar results have been observed in ligniosus bean (Prodhan and Bari, 2001). The outer wall of the epidermis of 2 days old root seems to be slightly thickened probably due to the formation of thin cuticle (Fig. 3). The cells of this stage are irregular, oval or slightly rectangular in shape. With ages the epidermal cells at the basal part gradually become regular as seen in 3 days old

root. The epidermal cells at this stage of growth become somewhat round or oval. The outer walls of the cells are thicker than the adaxial and lateral walls. The size of the cell has been considered by diameter and not by length. Along with this age, the epidermal cells gradually become more or less tangentially flattened (Fig. 3). The abaxial walls of the epidermis become more thickened. The epidermal cells have been found to be more regular in shape. Along with the age, the epidermal cells, both small and large, become smaller in size. The epidermis becomes disrupted here and there of older roots. This is probably due to stress of secondary growth and sharp increase in girth.

Cortex

There are 12-16 layers of cortical cells in the root of country bean (Fig. 3). There are 8-12 layers of cortical cells in the root of lignosus bean (Prodhan and Bari, 2001). The basal part contains comparatively less number of cortical layers while the apical part contains more. The cortical cell of 6 days old root is radially 14-16 layered in the apical part and 12-14 layered in the upper part (Fig. 3). The young cortical cells contain small intercellular spaces. As the radical elongates the diameter of the root decreases and size of the cortical cells becomes smaller but the number of cortical layers remains more or less same. The number of layers of cortical cells slightly increases particularly at the basal part of later stage of primary growth. The number of intercellular spaces increases along with the age. All the cells of the cortex become somewhat round, oval or polygoual in shape as seen in 2 days old root (Fig. 1). 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 of the cortex at one side (radially 2-3 cells) near the stele are hyperchromatic in nature (Fig. 4). No tanniniferous cells have been found during present investigation. Similar results have been observed in lignosus bean (Prodhan and Bari, 2001). As the diameter of the 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 protostele (Fig. 1). Four poles of xylem and four poles of phloem appear in the hypocotyl- root axis of one-day-old root. One strand of xylem alternates with one strand of phloem of the basal part of 2 days old root (Fig. 1). The initiation and development of treachery sieve elements in the germinating seeds and mature dry seeds have not been studied. The poles of both xylem and phloem extend towards the distal part as the radicle elongates. Adaxial to protoxylem, metaxylem begins to differentiate rapidly (Fig. 1 and 2). Gradually the centre is filled up with big metaxylem vessels (Fig. 3). The cambium initiates in the root of 4 days old seedling and begins to cut off secondary tissues within 2-3 days.

Primary Xylem

There are 4 poles of xylem in 2 days old root (Fig. 1), which are well apart from each other. In the apical part of the root, each xylem pole 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 basal 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. The immature vessel members show shrunken protoplasm and some secondary thickening in the wall. There are large thin walled parenchymatous cells at the centre of the root, which is known as pith (Fig. 1). 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. Each pole

Fig 1 T S through the basal part of the root of a 2 days old plant showing cortex (cor), 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 X 290

Fig 2 T S of the basal part (near hypocotyl) of the root of a 5 days old plant showing cortex (cor), xylem (x), phloem (ph) and pith (p) There are 4 strands of xylem and 4 strands of phloem One strand of xylem alternates with one strand of phloem Metaxylem is continuing to form in the centre X 290

generally contains 16-20 vessel members of which 10-13 is mature in the basal part of 2 days old root (Fig. 1). The vessels are more or less round, oval, hexagonal or polygonal in shape with prominent secondary thickening. The bigger vessels are adaxial while the smaller ones are abaxial. Subsequently more elements differentiate near these vessels on either side of the axis of 4 poles of protoxylem. Gradually the centre is filled up with big metaxylem vessels (Fig. 3). No secondary growth has been observed in the primary root till it is 6 days old.

Primary Phloem

There are 4 poles of phloem in 2 days old root (Fig. 1), which are well apart from each other as compared to the xylem poles. Immature sieve elements are present in the hypocotyl-root axis. Each pole consists of protophloem and metaphloem sieve elements. In the apical 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 basal part, it contains one mature protophloem and more metaphloem sieve elements. In the basal part, the metaphloem consists of both mature and immature sieve elements. The root apex does not contain any mature or differentiating sieve elements. The protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma without any companion cell. 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 5 days old roots (Fig. 2). Some of the metaphloem sieve tubes contain companion cells in the basal part of the root. There are groups of fibre cells in the primary phloem region of 8 days old root (Fig. 4). The tannin cells have been observed in the phloem region of the younger root.

Cambium

The cambium has been found in the basal part of 4 days old root. Gradually it extends towards the apical part. Within 2-3 days of initiation, the cambium forms a ring and begins to form secondary tissues (Fig. 3). The cambium is active in all places of the ring except the abaxial side of 4 poles of xylem tissues (Fig. 3). The cambium produces secondary xylem adaxially and secondary phloem abaxially. The cambium zone consists of several layers of tangentially flattened compact cells. 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 basal part of 6-7 days old root (Fig. 3). The cambium gives rise to different elements of secondary xylem and ray cells on its adaxial side. The mature secondary vessel members have been found in 8-10 days old root. They are fully devoid of protoplasm at this stage and are mostly polygoual, oval or round in shape with prominent secondary thickening (Fig. 4). The secondary xylem continues to form with the age. Lots of xylem vessels have been found in the mature root (Fig. 5 and 6). The vessel members are large, medium and small in size. The number of large vessel is more in comparison to that of small vessels (Fig. 5). Most of the vessels are scattered and the others are radially arranged (Fig. 5). Most of the vessels are solitary while the others are paired or multiple (Fig. 5). The spaces between the vessels are filled up mostly with parenchyma and fibre cells. The fibre cells are thick walled with small lumen. The ray cells are arranged radially and is also thickened to some extend. There are thick walled axial parenchymas around and in between the vessels. Most of the axial parenchyma is round or oval. The number of ray parenchyma is more than the axial parenchyma. The ray parenchyma is radially elongated. The structures of secondary xylem in the root of country bean are similar to those of many slightly woody herbaceous dicotyledonous plants (Islam *et al*., 2003, 2005; Prodhan and Bari, 2001; Prodhan and Haque, 1986). The elements of primary xylem both protoxylem and metaxylem remain intact near the centre.

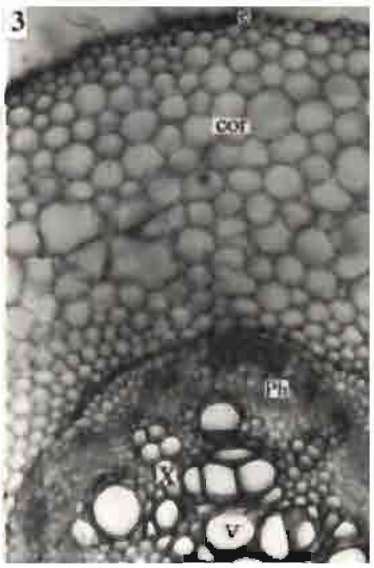

Fig 3 T S of the basal part of the root of a 6 days old plant showing epidermis (e) with root hairs, cortex (cor), phloem (ph) and xylem (x) The centre is filled up with metaxylem vessels (v) X 290

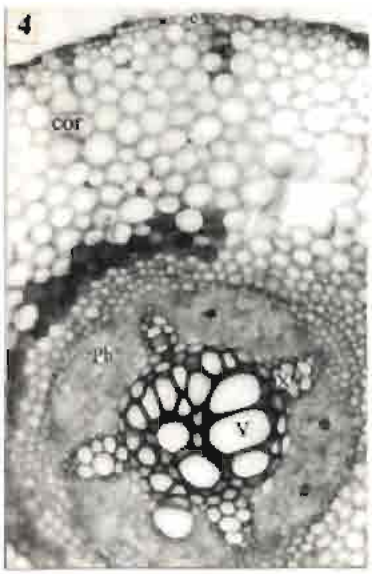

Fig 4 T S through the middle part of the root of a 8 days old plant showing epidermis (e), cortex (cor), phloem (ph) and xylem (x) The centre is filled up with metaxylem vessels (v) Xylem pole shows protoxylem vessels Phloem pole shows mature and immature sieve tube elements and fibre cells Hyperchromatic cells are present adaxial to the cortex X 290

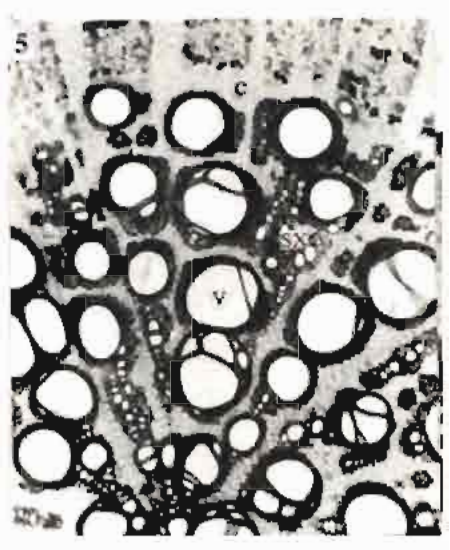

Fig 5 T S of the root of a mature plant showing cambium (c), secondary phloem (sp), secondary xylem (sx) with large and small secondary xylem vessels (v) There are lots of axial and ray parenchyma and few fibre cells. X 140

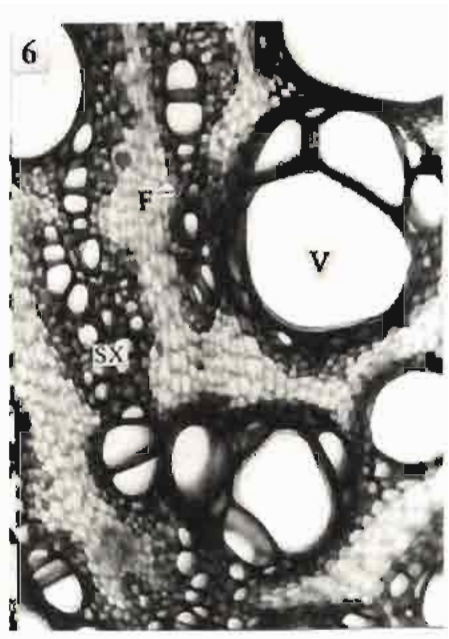

Fig 6 T S of the root of a mature plant showing secondary xylem (sx) secondary xylem vessels (v) Vessels are large and small There are lots of axial and ray parenchyma and few fibre cells (F) X 290

Fig 7 T S of the root of a mature plant showing cambium (c), secondary phloem (sp), secondary xylem (sx), secondary xylem vessels (v) and periderm (Pr) Secondary phloem shows several groups of phloem fibres and parenchyma cells X 132

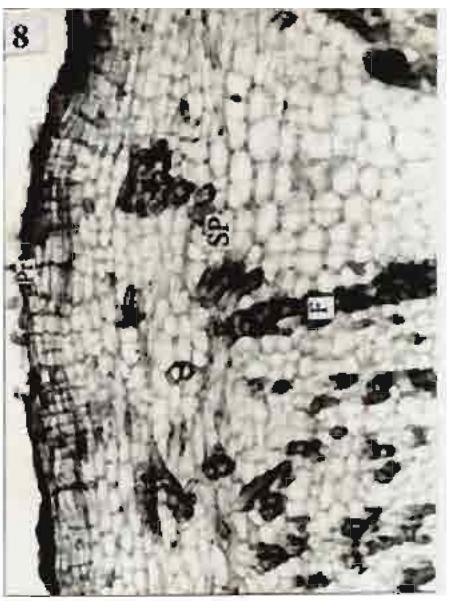

Fig 8 T S of the root of a mature plant showing secondary phloem (sp) and periderm (Pr) Secondary phloem shows several groups of phloem fibres (F) and parenchyma cells Periderm shows cork cells, phellogen and phelloderm X 280

Secondary Phloem

The secondary phloem begins to form in the basal part of 6-7 days old root (Fig. 3). 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 plants are mostly primary in origin. Several strands/poles of secondary sieve elements have been found in 6-8 days old root (Fig. 3 and 4). The sieve elements are at various stages of development at this stage. The phloem zone is narrow consisting of 6-8 layers of cells of 8 days old root (Fig. 4). Phloem fibers are found in the primary phloem region. The fibre cells are thick walled with small lumen. The number of sieve elements increases with the age of the plant. Lots of secondary phloem fibers are present in the mature root (Fig. 7 and 8). The fibre cells are arranged in groups. In each group there are 10-15 cells. The fibre groups/poles are radially arranged in such a way that the structure seems to be a pyramid (Fig. 7). Lots of parenchyma cells are present in between the pyramids and the fibre groups (Fig. 7 and 8). Adaxial to the phloem region, tanniniferous cells have been observed in the mature root (Fig. 7).

Periderm

The periderm normally forms in the root of country bean (Fig. 7 and 8). The epidermis ruptures here and there and the cells gradually disorganized. After a partial or total disintegration of the epidermis the phellogen appears. It appears in the cortex, 4-5 layers beneath the epidermis. The phellogen produces 4-6 layers of cork cells abaxially and 2-3 layers of phelloderm adaxially. The cork cells are apparently devoid of protoplasm and are thick walled. They are tangentially flattened and brick shaped in appearance (Fig. 8).

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