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Stem Anatomy of Country Bean

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Abstract: Anatomical investigation has been made on the stem of country bean (*Lablab purpureus* (L.) Sweet) at different stages of growth following the standard paraffin method of microtechnique. The epidermis is single layered with multicellular hairs and glandular trichomes. Beneath the epidermis there are 5-10 layers of cortical cells. The primary vascular tissue appears after the elongation of the first internode of the stem. The vascular bundles are collateral and arranged in a ring. There are two types of vascular bundles, large and small. There are one or more 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. The pericycle is discontinuous. Two adjacent groups of sclerenchyma are connected by one or two layers of sclerenchymatous cells. The cambium initiates in the primary vascular bundle between xylem and phloem at the basal part of the stem of 4 days old plant. Gradually it extends towards the upper part. The cambium is at first confined to the fascicular region. Subsequently it extends into the interfascicular region forming a complete cambial ring. After the formation of the fascicular cambium it gives rise to the secondary xylem adaxially and secondary phloem abaxially. In the mature stem, most of the vessels are multiple, some are paired while the others are solitary. Most of the fibre cells in the phloem region are found in groups. The fibre cells are arranged in such a way that the structure looks like a pyramid. Tannin cells are present in the phloem region of younger and mature stem. The secretory cells devoid of tanniniferous contents have been observed in the secondary phloem region of the mature stem. The phellogen appears in the deeper cortex and produces periderm with lenticel. The periderm consists of 3-5 layers of cork cells abaxially and 2-3 layers of phelloderm adaxially.

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

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 (Verdcourt, 1970; Zeven and De Wet, 1982). 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 (Bailey, 1949). 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 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 as "dal" 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 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* (Bari, 2000) but no work has been carried out with *Lablab purpureus*. The development and structure of different tissues of cowpea (Begum, 2001); *Sesbania rostrata* (Prodhan and Sarkar, 2002); *S. sesban* (Sarkar and Prodhan, 2001); *S. formosa* (Hossain, 1997) and *Cajanus cajan* (Bisen and Sheldrake, 1981; Hossain, 1999) have been investigated. Information on the gross and developmental anatomy of different tissues of *Lablab purpureus* is lacking. Therefore, the present research work has been undertaken to investigate the anatomical features of the stem 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 (Bari and Prodhan, 2001a, b). Some seeds were also placed on moist filter paper in petri dishes in the laboratory at room temperature of about 26-28^{0}C. The petri dishes were kept in dark for about 24 hrs. The sprouting was considered as the zero hour of age of the plant (Bari and Prodhan, 2001a, b; Prodhan and Bari, 2001). For investigation, the plant samples were collected from the petri dishes, pots and plots 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; Haque and Prodhan, 1987). The materials fixed in FAA were washed in running water for 2-4 hrs 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 (Bari and Prodhan, 2001a, b; Haque and Prodhan, 1987; 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 (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: There is a single layer of epidermis in the stem of country bean (*Lablab purpureus* (L.) Sweet). The epidermis consists of small and large cells. Both small and large cells are irregularly distributed throughout the epidermis (Fig. 1, 2, 4, 6). The epidermal cells are more or less round, oval, polygonal or irregular in shape (Fig. 2, 4, 6). Similar types of epidermal cells have been reported in the stem of lignosus bean (Bari and Prodhan, 2001b). The epidermis bears multicellular hairs and glandular trichomes (Fig. 2, 6, 12). The glandular trichomes are multicellular. Similar types of trichomes and hairs have been reported in the stem of lignosus bean (Bari and Prodhan, 2001b) and pigeonpea plants (Bisen and Sheldrake, 1981). In Phaseoleae, the hairs are glandular type and club-shaped with or without a distinct stalk (Metcalfe and Chalk, 1950). The abaxial, adaxial and lateral walls of the epidermal cells are more or less uniformly thick as seen in transverse sections of the middle and upper parts of the stem of 4 days old plant (Fig. 1). The epidermal cell walls of the stem have been found to be thicker than those of the root (Islam, 2002). According to Esau (1965) the cell walls of the epidermis may vary in different parts of the plant. The epidermal cells become tangentially flattened as seen in the older stem (Fig. 14). After considerable thickening of the abaxial wall a thin layer of cuticle is formed over the epidermis (Fig. 2). A thin cuticle has been observed in the stem of lignosus bean (Bari and Prodhan, 2001b) and pigeonpea (Hossain, 1999). The cuticle becomes thickened along with the age of the plant. The cuticularization depends on the type of the plant and the environment where it grows (Cutter, 1978; Esau, 1965, 1977; Fahn, 1967). The epidermis ruptures and becomes disrupted here and there as seen in older stem. This is probably due to the stress of secondary growth and sharp increase in girth.

Cortex: There are 5-10 layers of cortical cells in the stem of country bean as seen in transverse sections (Fig. 1, 3, 4, 6, 11, 14). Bari and Prodhan (2001b) have reported 5-9 layers of cortical cells in the stem of lignosus bean. The cortex is composed of large and small cells. The abaxial and adaxial cells of the cortex are smaller than that of the middle region (Fig. 1, 3, 4, 5). Most of the cells of the cortex are round, oval or polygonal in shape while others are irregular. The abaxial layer of the cortex is more or less similar to that of the epidermis as seen in the stem of 12 and 14 days old plant (Fig. 8, 9, 10, 11). There are small

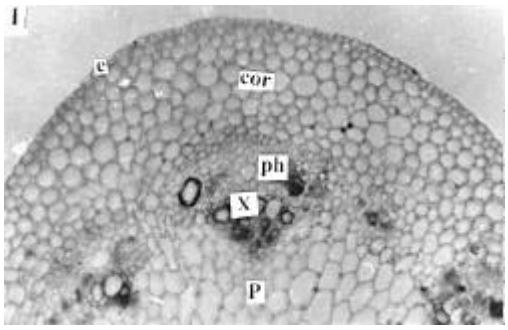

Fig. 1: T.S. of the middle part of the stem of a 4 days old plant showing epidermis (e), cortex (cor), phloem (ph), xylem (x) and pith (p). X 260

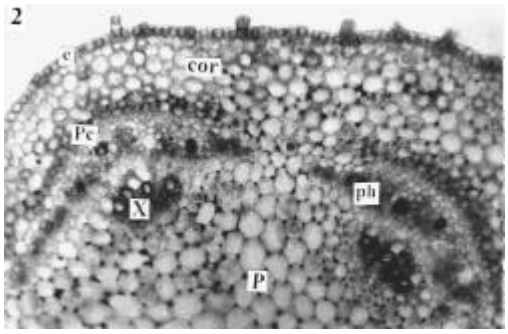

Fig. 2: T.S. of the basal part of the stem of a 4 days old plant showing epidermis (e) with glandular trichomes, cortex (cor), phloem (ph), xylem (x), discontinuous pericycle (pe) and pith (p). X 260

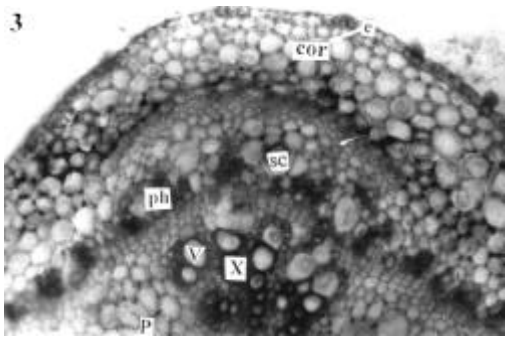

Fig. 3: T.S. of the middle part of the stem of a 6 days old plant showing epidermis (e) with cuticle, cortex (cor), small and large vascular bundles, phloem (ph), xylem (x), xylem vessel (v), secretory cell (sc) and pith (p). X 260

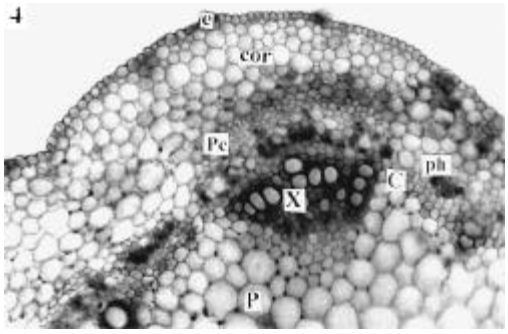

Fig. 4: T.S. of the middle part of the stem of a 7 days old plant showing epidermis (e) with thin cuticle, cortex (cor), large and small vascular bundles, bundle cap or discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x) and pith (p). Epidermal circumference is slightly wavy. X 280

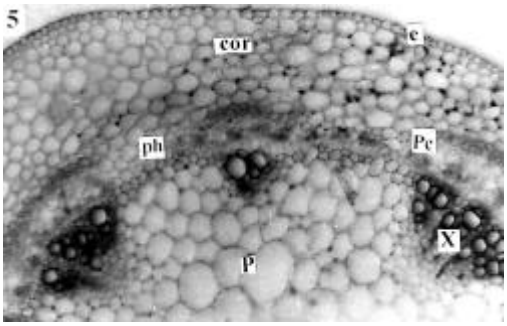

Fig. 5: T.S. of the apical part of the stem of a 8 days old plant showing epidermis (e) with cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle (pc), phloem (ph), xylem (x) and pith (p). X 260

and large intercellular spaces in the cortical parenchyma. With the age the intercellular spaces become prominent. The stem is not completely round but slightly ridged or wavy. In the older stem there are small ridges and shallow furrows. The number of cortical layer is more in the ridge and less in the furrow (Bari and Prodhan, 2001b; Haque and Prodhan, 1987; Metcalfe and Chalk, 1950). The number of cortical layer varies according to the age, size and level of secondary growth of the plant or plant parts. The cortical cells of the basal region of the stem have been found to be smaller than the upper region as seen in transverse sections of 8 days old plant (Fig. 5). At the upper part of the stem, the abaxial 1-2 layers of cortical cells are smaller in size, comparatively thick walled with

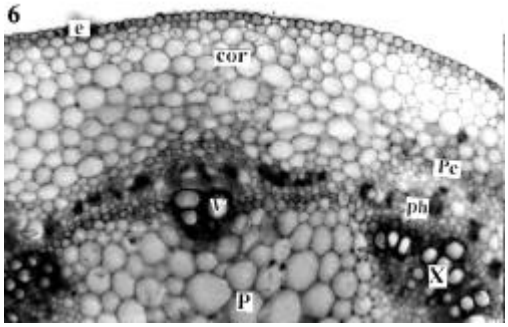

Fig. 6: T.S. of the middle part of the stem of a 10 days old plant showing epidermis (e) with cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle (pc), phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260

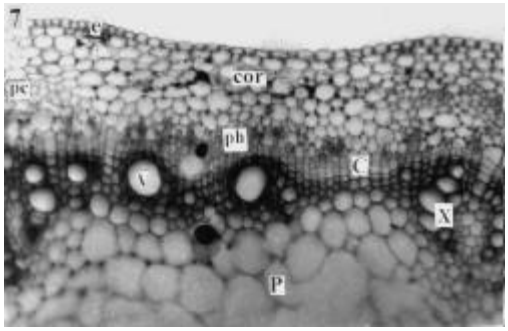

Fig. 7: T.S. of the basal part of the stem of a 10 days old plant showing epidermis (e), cortex (cor), discontinuous pericycle (pc), cambium (c), primary and secondary phloem (ph), primary and secondary xylem (x), xylem vessel (v) and pith (p). Cambium forms a continuous ring. X 265

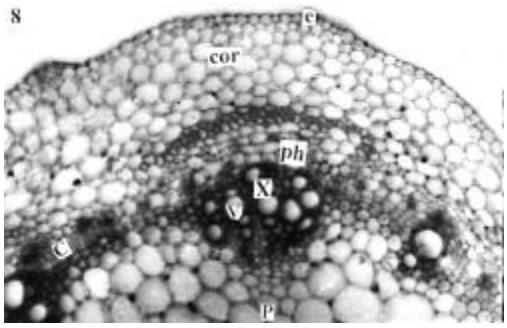

Fig. 8: T.S. of the apical part of the stem of a 12 days old plant showing epidermis (e) with cuticle, cortex (cor), vascular bundles, discontinuous pericycle, phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260

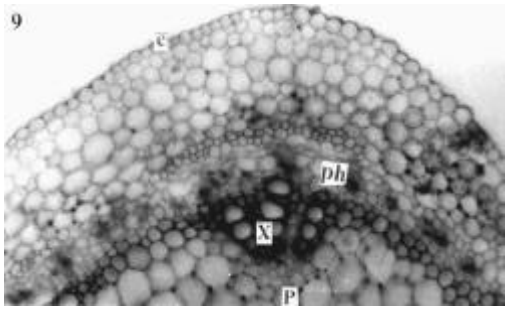

Fig. 9: T.S. of the middle part of the stem of a 12 days old plant showing epidermis (e), phloem (ph), xylem (x) and pith (p). X 290

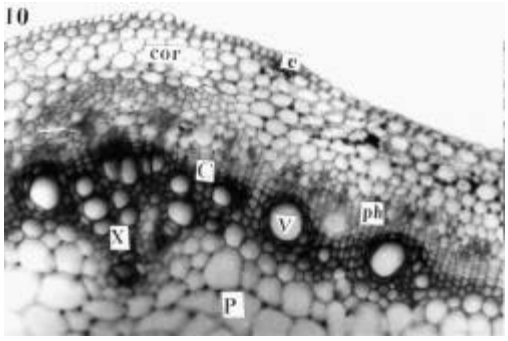

Fig. 10: T.S. of the basal part of the stem of a 12 days old plant showing epidermis (e) with cuticle, cortex (cor), vascular bundle with bundle cap, cambium (c), phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260

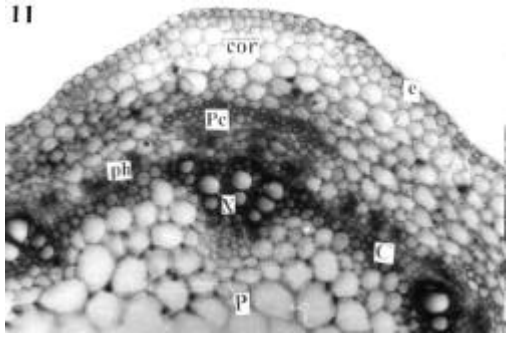

Fig. 11: T.S. of the middle part of the stem of a 14 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x) and pith (p). X 260

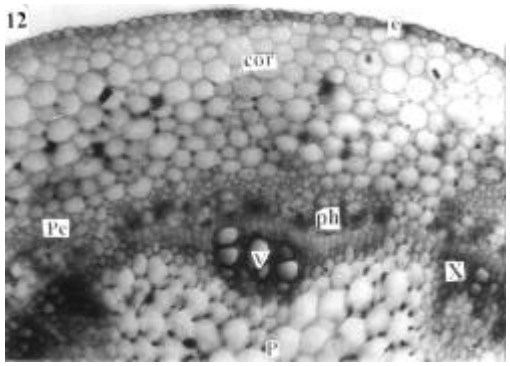

Fig. 12: T.S. of the apical part of the stem of a 16 days old plant showing epidermis (e) with thin cuticle and glandular trichomes, cortex (cor), large and small vascular bundles, bundle cap or discontinuous pericycle (pc), cambium, phloem (ph), xylem (x), xylem vessel (v) and pith (p). X 260

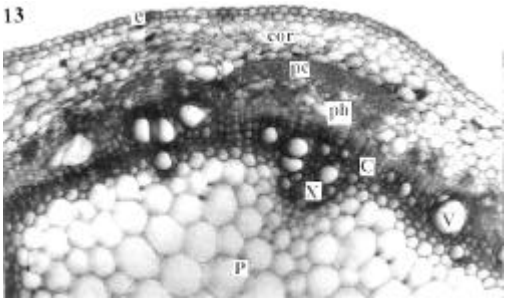

Fig. 13: T.S. of the middle part of the stem of a 16 days old plant showing epidermis (e) with thin cuticle, cortex (cor), large and small vascular bundles, discontinuous pericycle (pc), cambium (c), phloem (ph), xylem (x) and pith (p). X 275

small intercellular spaces as seen in transverse sections of 8, 12 or 16 days old plant. There are little or no intercellular spaces between the outermost layer of the cortex and the epidermis as seen in transverse sections of 12 days old plant (Fig. 8, 9, 10). Similar result has been reported for the stem of lignosus bean (Bari and Prodhan, 2001b).

The outermost layer of the cortex contains chloroplasts. No endodermis has been found in the stem of country bean during the present investigation. The cortical cells become tangentially flattened in the older or mature stem. The cortical cells of the stem of country bean have been found to be smaller than that of the hypocotyl as seen in transverse sections (Islam, 2002). The tanniniferous cells have not been found in the cortical region of the stem during the present investigation.

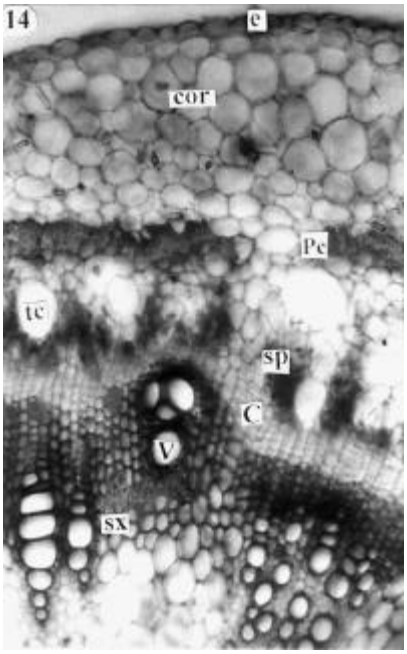

Fig. 14: T.S. of the basal part of the stem of a 25 days old plant showing epidermis (e) with cuticle, cortex (cor), discontinuous pericycle (pc), cambium (c), secondary phloem (sp), secondary xylem (sx), xylem vessel (v) and tannin cells (tc) without content. X 290

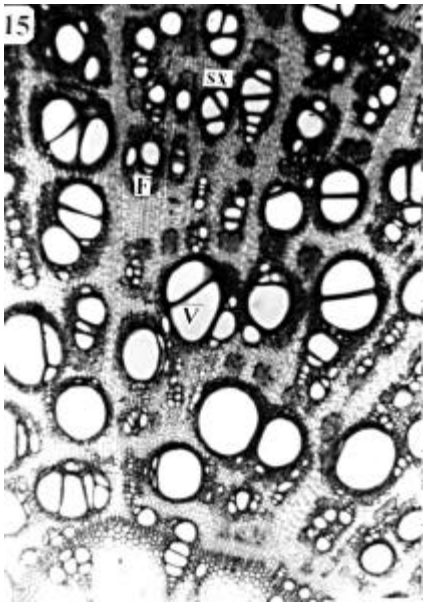

Fig. 15: T.S. of the stem of a mature plant showing secondary xylem (sx), xylem vessel (v), xylem fibres (F), ray and axial parenchyma. X 132

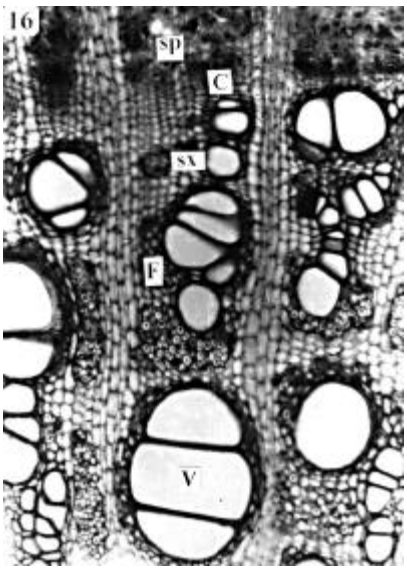

Fig. 16: T.S. of the stem of a mature plant showing secondary phloem (sp), cambium (c), secondary xylem (sx), secondary xylem vessel (v), xylem fibres (F), ray and axial parenchyma. X 280

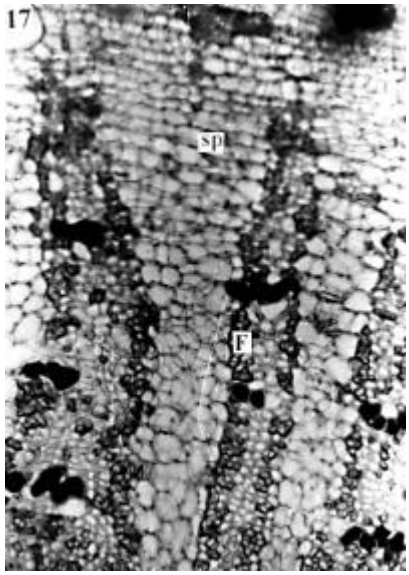

Fig. 17: T.S. of the stem of a mature plant showing secondary Phloem (sp), pholem fibres (F), ray and axial parenchyma. X 290

Bari and Prodhan (2001b) have reported tannin cells in the cortex of the younger hypocotyl but not in the stem of lignosus bean. Tannin cells have been reported in the cortex of the stem of *Sesbania rostrata* (Prodhan and Sarkar, 2002) and *Sesbania sesban* (Sarkar and Prodhan, 2001). The abaxial cells of the cortex are ruptured

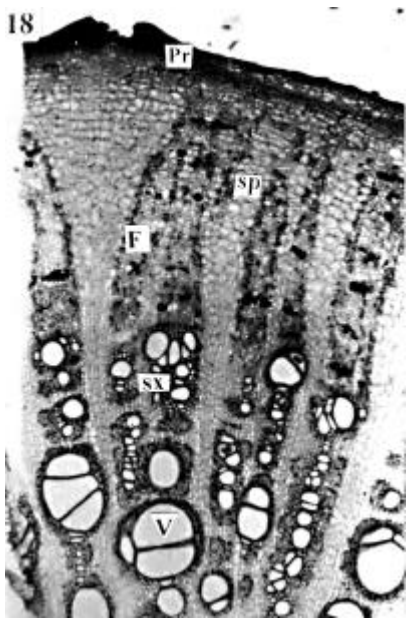

Fig. 18: T.S. of the stem of a mature plant showing periderm (Pr) with lenticel, cambium, secondary xylem (sx), xylem vessel (v), secondary phloem (sp), phloem fibres (F), ray and axial parenchyma. A few secondary xylem vessels remain in paired. X 132

and broken here and there and disorganized in the later stage of growth.

Primary vascular tissue: The primary vascular tissue appears after the elongation of the first internode of the stem. The internode between cotyledonary node and first leaf is considered as the first internode of the stem. It forms for a short period as the cambium appears soon. The arrangement of vascular tissues is collateral as seen in transverse sections in the first internode of the stem of 4 or 6 days old plant (Fig. 1, 2, 3). The vascular bundles are arranged in a ring. There are two types of vascular bundles, large and small (Fig. 1, 3, 4, 5, 6, 8, 9). There are one or more small vascular bundles in between two large bundles (Fig 5, 6, 8). The pericycle is discontinuous in the stem of country bean (Fig. 5, 6, 11). The discontinuous pericycle is also found in the hypocotyl of country bean (Islam, 2002). Similar result has been reported for the hypocotyl and stem of lignosus bean (Bari and Prodhan, 2001a, b) and for the stem of pigeonpea (Hossain, 1999). A continuous ring of sclerenchyma constituting the pericycle has been reported for pigeonpea stem (Bisen and Sheldrake, 1981). Radially each group of sclerenchyma consists of more or less 2-4 cells. Two adjacent groups are rarely connected by one or two layers

of sclerenchyma. Sometimes 2-3 or more vascular bundles, either large or small, contain a single band of sclerenchyma on their abaxial sides (Fig. 5). The cells of the pericycle contain prominent secondary thickening and small lumen. Such sclerenchymatous cells have different origin in different plants (Esau, 1965, 1977; Fahn, 1967; Pandey, 2001). So, the origin of this sclerenchymatous tissue of country bean needs a thorough investigation.

Primary xylem: The large vascular bundle contains xylem and phloem but the small vascular bundle may or may not contain both xylem and phloem (Fig. 1, 3, 4, 5, 8). The primary xylem develops only for a short period. Many vessel members are present in the large vascular bundle but a few vessel members have been found in the small vascular bundle (Fig. 1, 3, 4). The vessels are small and large. The size of the cells have been considered by diameter and not by length. Most of the vessel members are radially arranged while the others are scattered in the vascular bundle. The smaller vessels are adaxial to the bigger vessels (Fig. 3, 4). The mature vessel is completely devoid of protoplasm and contains thick secondary wall with large lumen. The vessel members are round, oval or polygonal in shape as seen in transverse sections. There are 6-10 vessel members in the large vascular bundle and 1-5 vessel members in the small vascular bundle (Fig. 3, 4, 5, 6). Some vessel members are paired while the others are solitary. The cells in between and around the vessel members are mostly of axial xylem parenchyma, other elements are primary xylem fibres. The primary tracheary elements are continued to form in the stem of country bean till the activity of the cambium continues. Similar result has been reported in the stem of lignosus bean by Bari and Prodhan (2001b). No secondary growth has been observed in the stem till the plant is 6 days old.

Primary phloem: There are several poles of primary phloem abaxial to the primary xylem (Fig. 1, 2, 3, 4). In the large bundle there are a number of sieve elements and phloem parenchyma in the phloem zone (Fig. 1, 2). Like primary xylem they mature rapidly. There may be only one mature or immature sieve element in the small vascular bundle. There are several poles of sieve elements in the younger stem. The number of phloem poles increases along with the age. The sieve elements are separated from each other by phloem parenchyma. Each pole consists of 1-4 sieve tube elements. In many cases sieve tubes have been found to accompany their companion cells. The sieve elements are also found to be scattered as seen in transverse sections of the stem of 4 or 5 days old plant (Fig. 1, 2). Some of the sieve elements are well apart and some are closer to each other. Similar findings have been reported for the hypocotyl and stem of lignosus bean (Bari and Prodhan, 2001a, b). The primary sieve elements are continued to form in the stem till the activity of cambium continues. No secondary growth has been observed in the first internode of the stem till the plant becomes 6 days old. The tannin cells have been found in the phloem region of the younger stem of country bean (Fig. 1, 2). The secretory cells devoid of tanniniferous contents have been observed in the phloem region of the stem (Fig. 14). Bari and Prodhan (2001b) have reported tannin cells in the phloem region of the younger stem of lignosus bean. Secretory cells or ducts have been reported in the phloem region at the apical part of the stem of *Sesbania formosa* (Hossain, 1997).

Cambium: The cambium has been found to initiate in the primary vascular bundle between xylem and phloem at the basal part of the first internode of the stem of 4 days old plant (Fig. 2) and gradually it extends towards the upper part. The cambial activity has been observed in the vascular bundle of the stem of 6 or 7 days old plant (Fig. 3, 4). Soon after the formation of the fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. The fascicular cambia in both large and small vascular bundles have been found to form simultaneously. The cambium is at first confined to the fascicular region of the primary vascular tissue. Subsequently it extends into the interfascicular region forming a complete cambial ring (Fig. 7, 10). Similar results have been reported by Bari and Prodhan (2001b), Haque and Prodhan (1987) and Metcalfe and Chalk (1950). The cambial ring becomes complete in the basal part of the stem of 10 days old plant (Fig. 6, 7). A continuous cambial ring in the stem of lignosus bean have been found in 9 days old seedlings (Bari and Prodhan, 2001b). After the formation of the cambial ring, the cambium becomes active in both abaxial and adaxial sides but it is more active on the adaxial side. In the active state of growth, the cambial zone consists of 4-6 layers of cells of cambial initials and their derivatives (Fig. 10, 14). The cells in the cambial zone are tangentially flattened and compact. The initials and the derivatives of the cambium in the stem have not been closely observed during the present investigation. The cambial activity seems to be more in the hypocotyl and stem compared to that in the root of country bean (Islam, 2002). The cambium has been found to remain active up to the senescence of the plant.

Secondary xylem: The secondary xylem has been found to form adaxially in the fascicular region after the formation of fascicular cambium at the basal part of the stem of 6 days old plant and gradually it extends towards

the upper part. It appears that the cambium becomes active in the fascicular region earlier than the interfascicular region (Fig. 6, 7). The fascicular cambium begins to form secondary tissues while the elements of primary vascular tissues are differentiating. After a level of secondary growth in the fascicular region, interfascicular cambium is formed and gives rise to the secondary tissues (Fig. 6, 7). Metcalfe and Chalk (1950) have reported that the cambium is at first confined to the primary vascular bundles and subsequently extends into the interfascicular region and gives rise to secondary tissues there. Similar result has been reported for lignosus bean (Bari and Prodhan, 2001b) and *Brassica campestris* (Haque and Prodhan, 1987). So at the early stage, the secondary growth is more in the fascicular region compared to that of the interfascicular region. Ultimately the secondary tissues become more or less equal in both the regions due to the vigorous activities of the interfascicular cambium (Fig. 14). After the formation of cambial ring the differentiation of secondary xylem takes place rapidly. The cambium gives rise to tracheary elements, xylem fibres and xylem parenchyma (both ray and axial).

The secondary xylem vessel begins to form adaxial to the cambium as seen in the lower part of the stem of 6 or 7 days old plant (Fig. 3, 4). The secondary xylem vessels are also found to form at middle and upper part of the first internode of the stem of 7, 8 or 10 days old plant (Fig. 4, 5, 6, 7). In the stem of lignosus bean the secondary xylem vessels have been found at 9 days old plant (Bari and Prodhan, 2001b). In the mature stem, some vessels are several times larger than the rest of the secondary xylem vessels as well as primary xylem vessels (Fig. 15, 16, 18). The size of the vessel is considered by diameter and not by length during the present investigation. Most of the vessels are distributed randomly and others are arranged radially as seen in transverse sections of young and mature stems. Some small vessels are arranged in between or among the big vessels while other small vessels are distributed randomly throughout the xylem region. Most of the vessels are multiple, some are paired and others are solitary as seen in mature stem (Fig. 15, 16, 18). Most of the paired vessels are radially arranged. In the stem of lignosus bean most of the paired vessels are radially arranged (Bari and Prodhan, 2001b).

The multiple vessels are composed of 3-6 members as seen in the transverse sections of mature stem (Fig. 15, 16, 18). Both large and small solitary vessels are round, oval or polygonal in shape while a few vessels are irregular in shape. However, all the paired and multiple vessels are irregular in shape. As much as 20-25 small vessels have been observed to be arranged radially in the xylem area of the mature stem (Fig. 18). The walls of both small and large vessels are thick and lignified. The mature vessels are devoid of protoplasm with prominent secondary thickening as seen in transverse sections (Fig. 15, 16, 18). The walls of the newly formed vessels are very thin and irregular in shape. 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 (Fig. 16). They are thick walled. The ray cells are small and large. Most of the ray cells in the xylem are multiseriate, few are biseriate and some are uniseriate and become gradually thickened. In the stem of lignosus bean most of the ray cells are uniseriate, few are biseriate and some are multiseriate (Bari and Prodhan, 2001b). The ray cells are larger than the axial parenchyma in the xylem region of the mature stem. The parenchyma (ray and axial) covers the major area of the secondary xylem.

The fibre cells are highly lignified, thick walled with small lumen. They are mostly pentagonal, hexagonal or square in shape as seen in transverse sections. Most of the fibre cells are arranged in groups and the rest are scattered. The fibre cells remain in between or around the vessels (Fig. 17, 18). The number of layers of secondary xylem increases in the stem along with the age of the plant. Lots of secondary xylem are found in the stem of mature plant. Similar results have been reported in the stem of lignosus bean (Bari and Prodhan, 2001b). The stem of the plant contains secondary xylem of considerable thickness. Different components of secondary xylem have not been studied thoroughly during the present investigation. Some attention has, however, been given on the vessel members, axial and ray parenchyma and fibres. 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 stem (Fig. 15).

Secondary phloem: The secondary phloem has been found to form abaxially in the fascicular region after the formation of fascicular cambium at the basal part of the stem of 6 days old plant and gradually it extends towards the upper part (Fig. 3). It appears that the cambium becomes active in the fascicular region earlier than the interfascicular region. So the secondary tissues are formed in the fascicular region much earlier than those in the interfascicular region. The fascicular cambium begins to form secondary tissues while the elements of primary vascular tissues are differentiating. After a level of secondary growth in the fascicular region interfascicular

cambium is formed and gives rise to the secondary tissues. According to Metcalfe and Chalk (1950) the cambium is at first confined to the primary vascular bundles and subsequently it extends into the interfascicular zone and gives rise to secondary tissues there. Similar results have been reported for lignosus bean (Bari and Prodhan, 2001b) and *Brassica campestris* (Haque and Prodhan, 1987). So at the early stage, the secondary growth is more in the fascicular region than that in the interfascicular region (Fig. 6, 7).

In the large vascular bundle there are a number of sieve elements and phloem parenchyma in the phloem zone. They mature rapidly like primary xylem. In the small vascular bundle there may be one or two mature or immature sieve elements. Before maturation of the sieve elements cambium becomes active and gives rise to the secondary phloem. It is difficult to distinguish primary sieve elements from secondary because these are more or less of same size and shape. Some phloem poles are present even where there are no corresponding xylem (Fig. 1, 3). Both primary and secondary sieve elements are well distributed outside the cambial ring (Fig. 7). Some sieve elements with their companion cells and axial phloem parenchyma have been found to form in the stem of 8 or 10 days old plant (Fig. 5, 6, 7). There are several poles of sieve elements in big vascular bundle. In one pole of sieve elements, there may be one or more sieve tube elements. The phloem zone is narrow. Radially it consists of 3-5 layers of cells in the big vascular bundle and 2-3 layers of cells in the small vascular bundle as seen in the stem of 6 or 8 days old plants (Fig. 3, 5). In the stem of lignosus bean, Bari and Prodhan (2001b) have reported 4-6 layers of cells in the big vascular bundle and 3-4 layers of cells in the small vascular bundle. The thickness of the phloem region increases as seen in the mature stem (Fig. 17, 18).

A number of parenchyma, both axial and ray, of secondary origin is present in the phloem zone. The axial parenchyma cells are larger than the ray cells as seen in transverse sections of the mature stem (Fig. 17, 18). Lots of secondary phloem fibres are present in the mature stem (Fig. 17, 18). Most of the fibre cells are arranged in groups. In each group, there are 10-15 cells. The fibre cells or groups are radially arranged in such a way that the structure looks like a pyramid (Fig. 17, 18). Lots of parenchyma cells (axial and ray) are present in between the pyramids and the fibre groups. Lots of tanniniferous cells have been found in the pyramid region of the secondary phloem of mature stem. The secretory cells devoid of tanniniferous contents have been observed in the secondary phloem region of the mature stem. The secondary phloem continues to form and the sieve elements remain active till the senescence of the plant.

Pith: The pith of the stem of country bean is prominent. The pith cells are thin walled with prominent intercellular spaces (Fig. 1, 2, 8, 9, 12). The pith is composed of small and large parenchymatous cells. The central cells of the pith are larger while the abaxial cells are smaller in size. The pith cells are round, oval, pentagonal, hexagonal or somewhat polygonal in shape as seen in transverse sections (Fig. 1, 2, 8, 9). Similar result has been reported in the stem of lignosus bean (Bari and Prodhan, 2001b). In primary structure of the stem, the pith increases in size due to the increase in diameter of the pith cells as well as the size of the intercellular spaces. The pith gradually decreases in size due to the continuous addition of secondary xylem. Similar findings have been reported for lignosus bean (Bari and Prodhan, 2001b) and *Brassica campestris* (Haque and Prodhan, 1987). Three to five 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 the older stem. Due to the stress of radial growth of the stem and the addition of secondary xylem towards the centre, the peripheral pith cells lose their intercellular spaces and become narrow (Esau, 1965) but the pith cells remain more or less unaffected at the centre as seen in the basal part of the older stem. In the upper region of the mature stem, the pith cells become disorganised, disintigrated and destroyed and thus the pith region becomes hollow.

Periderm: The well developed periderm has been observed in the basal part of the stem of country bean (Fig. 18). 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 mature stem (Fig. 18). Similar result has been reported in the stem of lignosus bean by Bari and Prodhan (2001b). The number of cork cells in a radial row depends on the age and size of the plant or plant parts (Cutter, 1978; Esau, 1965, 1977; Fahn, 1967; Pandey, 2001)). In *Sesbania* of Papilionaceae, a well developed periderm has been reported (Hossain, 1997; Prodhan and Sarkar, 2002; Sarkar and Prodhan, 2001). The development and morphology of different components of periderm have not been studied during the present investigation. In the stem of country bean, the periderm consists of 3-5 layers of cork cells and 2-3 layers of phelloderm with a narrow zone of differentiating phellogen. A well developed periderm has been found in the stem of

lignosus bean which consists of 4-5 layers of cork cells and 3-4 layers of phelloderm (Bari and Prodhan, 2001b). The periderm develops one after another from deeper cortex and as a result cortex is eliminated due to the formation of periderm. Similar results have been reported for lignosus bean (Bari and Prodhan, 2001b) and *Sesbania* (Hossain, 1997; Prodhan and Sarkar, 2002; Sarkar and Prodhan, 2001). Generally, the periderm is formed in the outer part of the cortical region of the stem (Cutter, 1978; Esau, 1965, 1977; Haque and Hossain, 1978). Lenticel has been observed in the periderm of the mature stem during the present investigation (Fig. 18).

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