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Anatomy of the Root of Pigeonpea (*Cajanus cajan*)

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Abstract: Anatomical investigation has been made on the root of pigeonpea (*Cajanus cajan* (L.) Millsp.) at different stages of growth following the standard paraffin method of microtechnique. The root of pigeonpea 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 primary xylem meet at the centre. Subsequently metaxylem forms near the centre on either side of the xylem strand. Ultimately the centre is filled up with big metaxylem vessels. The epidermis is single layered with root hairs and glandular trichomes. There are 8-13 layers of cortical cells in the root of pigeonpea. The cambium appears in the basal part of the root of 3-4 days old plant. Gradually it extends towards the root apex. The activity of cambium is similar to that of woody dicotyledonous herb. In the mature root, most of the vessels in the secondary xylem are solitary while the others are paired or multiple. The fibre cells in the phloem are arranged in groups. The fibre groups are radially arranged in such a way that the structure seems to be a pyramid. The epidermis is ruptured here and there, and the epidermal cells are disorganized due to the stress of secondary growth. Periderm is formed in the root one after another as the root increases in diameter.

Key words: Pigeonpea, *Cajanus cajan*, anatomy, root

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) belongs to the sub-family Papilionaceae under the family Leguminosae. It is mainly a subsistence crop in the tropics and sub-tropics. It is able to tolerate drought condition during the dry season. It is commonly grown for its dry split seeds which have a protein content of approximately 20-25%. In Africa and Central America, whole dry seeds without seed coats are cooked along or together with meat. The pigeonpea is grown as a field crop in Asia, mainly for the ripe seeds, green pods as vegetables and fodder (Purseglove, 1974). Pigeonpea as a multipurpose crop, is gaining popularity to the farmers of Bangladesh day by day.

In Bangladesh some attention has been given for the improvement of pigeonpea. For a successful improvement program it is the pre-requisite to know about the status of the plant in respect of its morphological, anatomical, genetical and physio-ecological features. Most of these biological phenomena of pigeonpea are known to some extent (Pursegloves, 1974) but the information on anatomical features of this plant is very scanty. Only some sporadic works have been carried out on the stem anatomy of pigeonpea (Bisen and Sheldrake, 1981; Hossain, 1999). Available literature shows that anatomical works on the root so far have been done with some papilionaceous plants, but no work has been carried out on the root of pigeonpea plant. Some developmental works have been carried out with the root of *Sesbania formosa* (Hossain, 1997), *Dipogon lignosus* (Prodhan and Bari, 2001), *Sesbania sesban* (Sarkar and Prodhan, 2001), cowpea (Begum, 2001), country bean (Islam, 2002), lentil (Hoque, 2002) and *Sesbania rostrata* (Prodhan and Sarkar, 2002). The development of different tissues, such as vascular tissues of the root of papilionaceae (Cutter, 1978; Esau, 1965, 1977; Haque and Engleman, 1978; Pandey, 2001) and periderm of the root (Cutter, 1978; Esau, 1965) has been investigated.

However, information on the gross and developmental anatomy of different tissues of the root of pigeonpea is lacking. Therefore, the present piece of research work has been undertaken to investigate the anatomical features of the root of pigeonpea (*Cajanus cajan* (L.) Millsp.) at different stages of growth.

Materials and Methods

Mature seeds of Pigeonpea (*Cajanus cajan* (L.) Millsp.) 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 July, 2001 and May, 2002. 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 root 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.

Roots of 1, 2, 3, 4, 6 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 6, 9, 10, 12, and 15 days old seedlings were collected from both polybags and pits of the plots. The roots of 15, 18, 21, 25 days old plants and mature plants were 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 h before dehydration. The materials fixed in Craff III were very succulent. They were dehydrated gradually making more grades of alcohol to avoid severe shrinkage (Ali *et al*., 1999; Haque and Prodhan, 1991; Prodhan and Haque, 1986; Bari and Prodhan, 2001a; Prodhan and Bari, 2001).

The dehydrated materials were gradually infiltrated with paraffin oil and low-melting-point paraffin wax (51°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°C). Repeated trial showed that there was less shrinkage when the materials were infiltrated for a longer period (Bari and Prodhan, 2001b; Haque and Prodhan, 1987; Prodhan and Haque, 1986; Sarwar and Prodhan, 2000). Serial transverse sections were made at 10-15 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 materials (Bari and Prodhan, 2001a,b,c; Mofazzel *et al*., 2002; Prodhan and Haque, 1986). Olympus binocular compound microscope (Japan) has been used to investigate the anatomical sections.

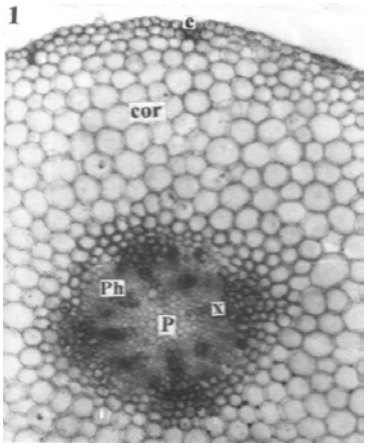

Fig. 1: T.S. of the basal part of the root of a 1 day old plant showing epidermis (e), cortex (cor), 4 poles of xylem (x) and 4 poles of phloem (Ph). Each pole of xylem contains protoxylem and metaxylem vessels and each pole of phloem contains mature and immature sieve elements and pith (P). x 260

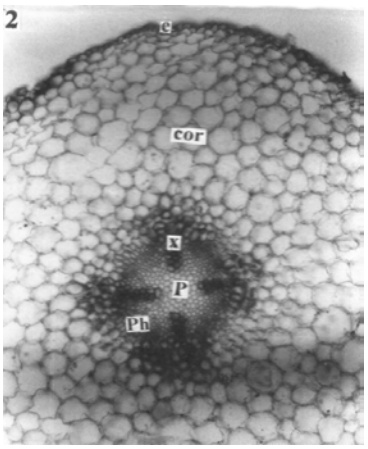

Fig. 2: T.S. of the basal part of the root of a 2 days old plant showing epidermis (e) with root hairs, cortex (cor), 4 poles of xylem (x) and 4 poles of phloem (Ph) and pith (P). x 260

Results and Discussion

Epidemris: The epidermis of the root of pigeonpea (*Cajanus cajan*) is single layered (Fig. 1, 2). The epidermal cells are regularly arranged as seen in transverse section of the root/radicle of one day old seedling (Fig. 1). The epidermal cells are more or less similar in size. Most of the cells towards the distal part of the root give rise to root hairs and glandular trichomes. The root hairs are unicellular, slender and elongated (Fig. 3, 4). Similar results have been found in lignosus bean (Prodhan and Bari, 2001), country bean (Islam, 2002), cowpea (Begum, 2001) and lentil (Hoque, 2002). The outer wall of the epidermis of 2 days old root seems to be slightly thickened probably due to the formation of a thin cuticle (Fig. 2). The cuticle is prominent in the 4 days old root (Fig. 4). The inner and lateral walls are more or less uniformly thick. As the root elongates, the cells in the basal region of the root gradually become regular and more or less tangentially flattened as seen in transverse

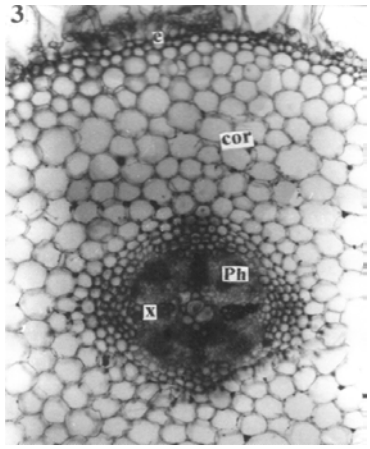

Fig. 3: T.S. of the basal part of the root of a 3 days old plant showing epidermis (e) with root hairs, cortex (cor), 4 poles of xylem (x) and 4 poles of phloem (Ph). The centre of the stele is filled up with metaxylem vessels. x 265

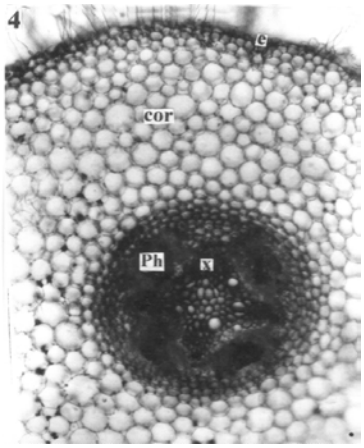

Fig. 4: T.S. of the basal part of the root of a 4 days old plant showing epidermis (e) with root hairs, cortex (cor), xylem (x) and phloem (Ph). The centre of the stele is filled up with big and small metaxylem vessels. x 260

sections of the root of 2 or 3 days old seedlings (Fig. 2, 3). Along with the age, the tangentially flattened cells gradually become more or less round, oval, polygonal or radially elongated. Similar results have been reported (Prodhan and Bari, 2001; Begum, 2001; Islam, 2002; Hoque, 2002). The abaxial walls of the epidermis become more thickened as seen in transverse section of 8-10 days old seedling (Fig. 8, 9). Along with the age epidermal cells become smaller. The epidermis becomes ruptured here and there in the older roots (Fig. 13). This is probably due to the stress of secondary growth and sharp increase in girth.

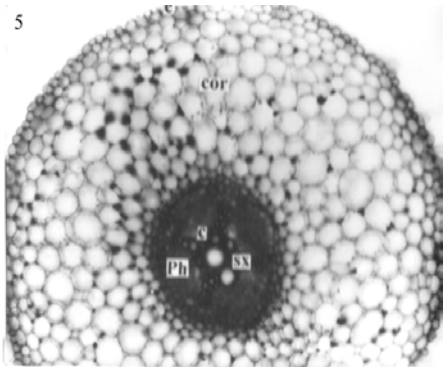

Fig. 5: T.S. of the middle part of the root of a 6 days old plant showing epidermis (e), cortex (cor), secondary xylem (Sx), phloem (Ph) and cambium (c). The centre of the stele is filled up with big and small metaxylem vessels. x 265

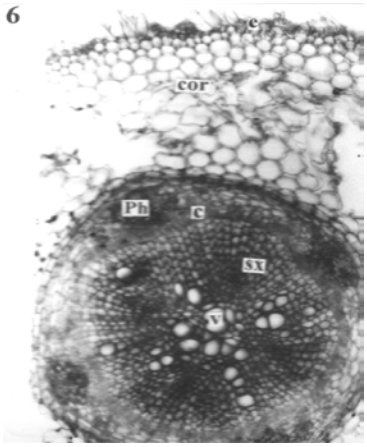

Fig. 6: T.S. of the basal part of the root of a 9 days old plant showing epidermis (e) with root hairs, cortex (cor), Secondary xylem (Sx) and phloem (Ph) poles and cambium (c). The centre of the stele is filled up with big and small metaxylem vessels (v). x 260

Cortex: There are 8-13 layers of cortical cells in the root of pigeonpea (Fig. 1-4). Prodhan and Bari (2001) have reported 8-12 layers of cortical cells in the root of lignosus bean whereas Begum (2001), Islam (2002) and Hoque (2002) have observed 7-10, 12-16 and 9-10 layers of cortical cells in the root of cowpea, country bean and lentil respectively. The lower part of the root of pigeonpea contains comparatively less number of cortical layers while the upper part contains more. All the cells of the cortex are somewhat oval, round or polygonal in shape as seen in 1-5 days old root (Fig. 1-4). The cells of middle layers of the cortex are larger in size than the abaxial and adaxial layers (Fig. 1-4). As the root elongates, the diameter of apical part decreases and the size of cortical

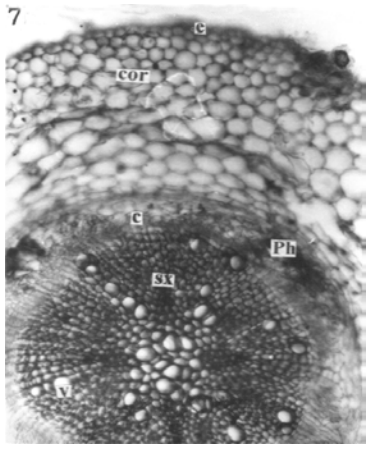

Fig. 7: T.S. of the basal part of the root of a 10 days old plant showing epidermis (e) with root hairs, cortex (cor), Secondary xylem (Sx) and phloem (Ph) poles and cambium (c). The centre of the stele is filled up with big and small metaxylem vessels (v). x 260

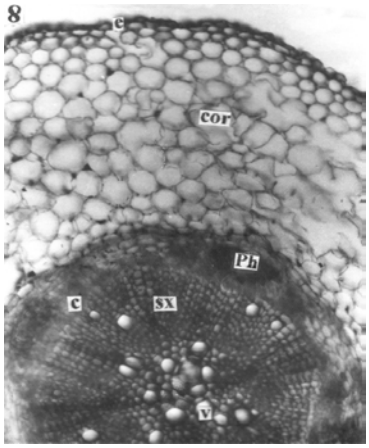

Fig. 8: T.S. of the basal part of the root of a 12 days old plant showing epidermis (e), cortex (cor), phloem (Ph), cambium © secondary xylem (Sx). The centre of the stele is filled up with big and small metaxylem vessels (v). x 265

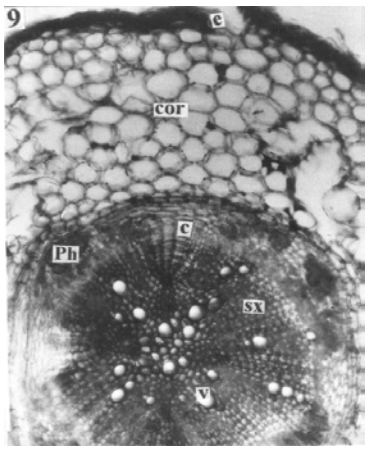

Fig. 9: T.S. of the basal part of the root of a 15 days old plant showing epidermis (e), cortex (cor), phloem (Ph), cambium (c), secondary xylem (Sx) and secondary xylem vessel (v). The centre of the stele is filled up with big and small metaxylem vessels (v). x 260

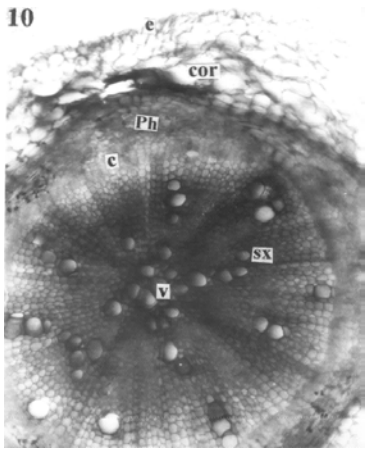

Fig. 10: T.S. of the basal part of the root of a 18 days old plant showing epidermis (e), cortex (cor), phloem (Ph), cambium (c), secondary xylem (Sx). Secondary xylem vessels (v) are scatteredly arranged. x 260

cells become smaller but the number of cortical layers remain more or less same as seen in transverse section. As the diameter of the root increases the cortical cells become tangentially flattened.

The cortical cells are thin walled with conspicuous intercellular spaces. The young cortical cells contain small intercellular spaces (Fig. 1-4). The number of intercellular spaces increases along with the age. One row of the cells gradually organizes around the stele to from endodermis at one day old seedling (Fig. 1). The cell walls of the endodermis are thicker than the cortical cells. The lateral walls of the endodermal cells are thicker than the abaxial and adaxial walls. The abaxial cells become ruptured and broken here and there, and disorganized due to the stress of secondary growth as seen in older roots (Fig. 13).

Primary vascular tissue: The primary root of pigeonpea shows a tetrarch protostele. Four poles of xylem and four poles of phloem appear in the basal part of the root of one day old seedling (Fig. 1). One strand of xylem alternates with one strand of phloem. The poles of both xylem and phloem extend towards the distal part as the radicle elongates. Adaxial to protoxylem, metaxylem begins to

differentiate rapidly with the increase of age (Fig. 1,2). Gradually the centre is filled up with big metaxylem vessels as seen in transverse sections of 3-6 days old seedling (Fig. 3-6). The pericycle is 2-3 layered with large cells on the abaxial side of each xylem pole and one layered in between them and arranged radially at the early stage of growth (Fig. 1-2). But at the later stage, the cells of the pericycle become smaller and more or less similar to that of endodermis and the layers become uniform around the stele. The cambium initiates in the root of 3-4 days old seedling (Fig. 3-4) and begins to cut off secondary tissues within 3-4 days (Fig. 5- 6).

Primary xylem: There are 4 poles of xylem in the root/radicle of 1 day old seedling. In the apical part of the root, each pole of xylem contains immature or developing protoxylem vessel. In the middle part, it contains mature protoxylem vessel and 1 or 2 metaxylem vessels while in the basal part, it contains protoxylem vessel and more metaxylem vessels (Fig. 1,2). The metaxylem in the basal part consists of both immature and mature vessels. The mature vessels are completely devoid of protoplasm and contain conspicuous secondary thickening in their cell walls (Fig. 1,2). The poles are apart from each other. The number of xylem vessels gradually increases in each pole with the increase of age. In the basal part of the root of 3 days old seedling each pole generally contains 10-14 vessel members of which 6-8 are mature (Fig. 3). The vessels are more or less round, hexagonal, or polygonal in shape with prominent secondary thickening (Fig. 3,4). 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 xylem and in the centre. Ultimately, the centre is filled up with big metaxylem vessels within 3-5 days (Fig. 3,4). Some of the subsequently formed vessels are big with large lumen and the others are small with small lumen and thick secondary walls. No secondary growth has been observed in the primary root till it is 5-6 days old. There are large thin walled parenchymatous cells, known as pith, at the centre of the basal part of the root of 1-2 days old seedlings as seen in transverse sections (Fig. 1,2).

Primary phloem: There are 4 poles of phloem in the root of 1 or 2 days old seedlings (Fig. 1,2). Each pole consists of protophloem and metaphloem sieve elements. In the upper part, each pole contains immature or differentiating sieve elements, 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 (Fig. 1,2). The metaphloem consists of both mature and immature sieve elements. The wall of the protophloem sieve elements stains more deeply than those of the surrounding cells. Similar results have been reported for country bean (Islam, 2002), cowpea (Begum, 2001), lignosus bean (Prodhan and Bari, 2001) and 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. In the root of lignosus bean, the protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma (Prodhan and Bari, 2001). In the root, the protophloem sieve element normally lacks companion cell (Esau, 1965). The 4 poles of phloem are well apart from each other compared to the xylem poles (Fig. 1,2). The number of sieve elements in each pole increases along with the age (Fig. 3,4). Several sieve elements have been found in each pole of phloem as seen in transverse section of 3-4 days old plants. The subsequently formed sieve elements are slightly bigger. The primary sieve elements are continued to form in the root till the plants are 6-7 days old after which secondary sieve elements begin to form.

Cambium: The cambium has been found to form in the basal part of the root of 3-4 days old seedlings (Fig. 3-4). Gradually it extends towards the tip. Within 2-3 days of initiation, the cambium forms a ring (cambial ring) and begins to form secondary tissues (Fig. 5). The cambium is more active in places between the xylem poles. The cambium produces secondary xylem adaxially and secondary phloem abaxially and it has been found to more active in its adaxial side. The cambial zone consists of several layers of tangentially flattened thin walled compact cells (Fig. 6-12). The cambium has been found to remain active till the senescence of the plant.

Secondary xylem: The secondary xylem begins to form in the root of 6 days old plant (Fig. 5). The cambium gives rise to different elements of secondary xylem and ray cells on its adaxial side. The differentiation of different cells derived from the 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 basal part of the 9-15 days old plant (Fig. 6-9). They are fully devoid of protoplasm at this stage. At the age of 8-15 days, the vessels are mostly concentrated in and around the centre of the root (Fig. 6-9). The vessels are mostly round, oval or polygonal in shape with prominent secondary thickening as seen in transverse sections (Fig. 6-13). The secondary xylem continues to form with the age. Lots of xylem vessels have been found in the mature root. The vessel members are large, medium and small in size (Fig. 10-13). The

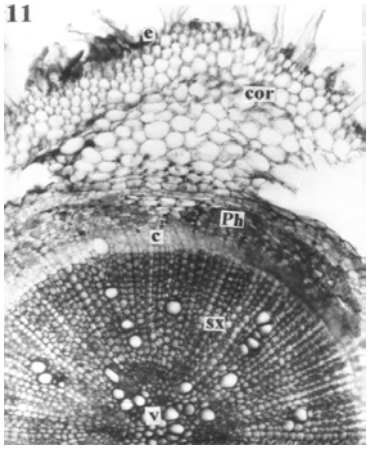

Fig. 11: T.S. of the basal part of the root of a 21 days old plant showing epidermis (e) with root hairs, cortex (cor), phloem (Ph), cambium © ring, secondary xylem (Sx) and secondary xylem vessels (v). x 260

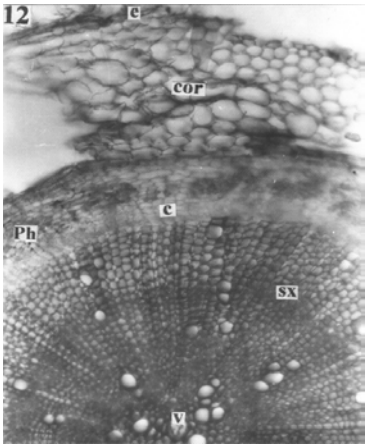

Fig. 12: T.S. of the basal part of the root of a 25 days old plant showing epidermis (e), cortex (cor), phloem (Ph), cambium © ring, secondary xylem (Sx) and secondary xylem vessels (v). x 260

number of large vessels is more in comparison to small vessels. Most of the vessels are scattered while the others are radially arranged (Fig. 10-13). Most of them are solitary and others are paired (Fig. 13,14). Similar result has been reported in *Sesbania sesban* (Sarkar and prodhan, 2001). The vessels are round, oval or polygonal in shape and some are irregular (Fig. 13,14).

The spaces between the vessels are filled up with parenchyma and fibre cells (Fig. 10-14). The fibre cells are thick walled with small lumen. The ray cells are arranged radially. They are uniseriate or multiseriate (Fig. 14). The ray parenchyma is thickened to some extent. There are axial parenchyma around the vessels and they are also thick walled (Fig. 13-14).

Fig. 13: T.S. of the root of a mature plant showing periderm (Pr) with lenticel (Lc), secondary phloem (Sp), cambium (c), secondary xylem (Sx) and secondary xylem vessels (v). Secondary phloem shows several groups of phloem fibres (F) and parenchyma cells. x 260

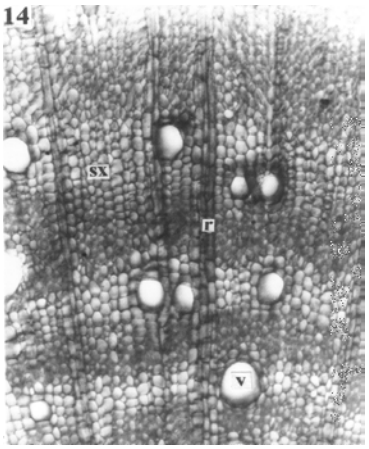

Fig. 14: Higher magnification of figure 13 showing secondary xylem (Sx) and secondary xylem vessels (v). Vessels are big and small. There are lots of axial and ray parenchyma (r) and few fibre cells. x 260

Secondary phloem: The secondary phloem begins to form in the basal part of the root of 6-8 days old seedlings. The secondary sieve elements have been found to form as an activity of cambium. The sieve elements of the root of 6-8 days old seedlings are mostly primary in origin as seen in transverse section. The poles of secondary sieve elements have been found in 8-9 days old root (Fig. 6). The secondary sieve elements are at various stages of development at this stage. In a single strand there may be one or more sieve elements (Cutter, 1978; Esau, 1965,

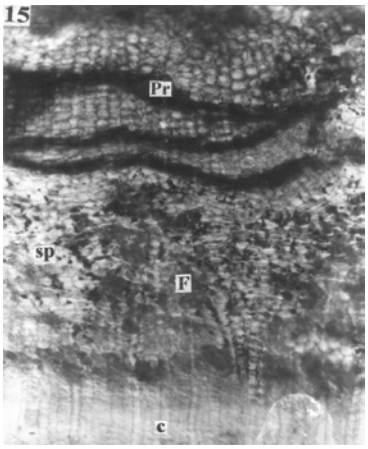

Fig. 15: Higher magnification of Fig. 13 showing cambium (c), secondary phloem (Sp) and layers of periderm (Pr). Secondary phloem (Sp) shows groups of phloem fibres (F) and parenchyma cells. x 260

1977; Pandey, 2001). 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 primary elements. No thorough study has been made on the ontogeny of the newly differentiated sieve elements during the present investigation.

The diameter of meta sieve tube members and the secondary sieve elements have been found to be more or less similar as seen in transverse sections. Therefore, it is difficult to distinguish primary and secondary sieve elements by size. The number of sieve elements increases with the age of the plant (Fig. 7-11).

Periderm: The periderm normally forms in the root of pigeonpea (Fig. 13). The epidermis ruptures here and there and the cells gradually disorganize. After a partial or total disorganization of the epidermis the phellogen appears. It appears in the deeper cortex. According the Esau (1965) the periderm normally originates in the root of dicotyledonous plant from a deep layer. The phellogen produces 4-5 layers of cork cells abaxially and 3-4 layers of phelloderm adaxially (Fig. 13-15). In the root of lignosus bean, the periderm consists of 3-5 layers of cork cells and 2-3 layers of phelloderm (Prodhan and Bari, 2001). The periderm consists of 4-6 layers of cork cells and 2-4 layers of phelloderm with a narrow zone of differentiating phellogen in the root of *Sesbania formosa* (Hossain, 1997). The cork cells are apparently devoid of protoplasm and are thick walled. The cork cells are tangentially flattened and brick shaped in appearance as seen in transverse section (Fig. 15). The periderm forms one after another as the root increases in diameter (Fig. 13-15).

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