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

[1]Shahanara Begum, [2]Md. Azharul Islam and [1]A.K.M. Azad-ud-doula Prodhan
[1]Department of Crop Botany, [2]Department of Entomology,
Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract: Anatomical investigation has been made on the stem of pigeonpea (*Cajanas cajan* (L.) Millsp.) at different stages of growth following the standard paraffin method of microtechnique. The vascular bundle of the stem are collateral and arranged in a ring. The cambium initiates in the primary vascular bundle between xylem and phloem at the basal part of the stem of 3 days old plant. After the formation of fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. Most of the vessel members are solitary and few are paired while others are multiple. The solitary vessel members are more in mature stem as compared to that of the younger stem. The well developed periderm was found in mature stem. The pith resembles a typical dicotyledonous stem.

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

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) belongs to the sub-family Papilionaceae under the family Leguminosae. It is one of the most important grain legume crop in tropics and sub-tropics. It is able to tolerate drought conditions during dry seasons. The dry split seeds which have a protein content of approximately 20-25%. Green pods are used as vegetables and fodder (Pursegloves, 1974). Pigeonpea is a multipurpose crop, is gaining popularity to the farmers of Bangladesh day by day.

Recently, some attention has been given for improvement of pigeonpea in Bangladesh. 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 a few works have been conducted with the mature stem anatomy of pigeonpea by Bisen and Sheldrake (1981). Available literature and some unpublished data shows that there are some sporadic works have been carried out on the stem anatomy of papilionaceous plants such as *Dipogon lignosus* (Bari and Prodhan, 2001a), cowpea (Begum, 2001), country bean (Islam, 2002), lentil (Hoque, 2002) and *Sesbania rostrata* (Prodhan and Sarkar, 2002) but no work has been carried out with the developmental anatomy of pigeonpea.

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

MATERIALS AND METHODS

The experiment was carried out in the university farm and as well as in the department of Crop Botany during the study period between July 2003 and May 2004. The seeds were sown in polybags were transplanted in pits of plots. Some seeds were also placed on moist filter paper in large petridishes in the laboratory at room temperature of about 26-28°C. The sprouting was considered as 0 h age of the plant.

Stems of 2, 3, 4, 6, 7 and 9 days old seedlings were fixed separately in Craf III (Sass, 1958) after making small pieces of about 5 mm in length. The stems of 9, 10, 12, 13 and 15 days old seedlings were collected from both polybags and pits of the plots. The stems of 15, 21, 25, 30 and mature plants were collected from the pits of the plots. These were fixed in FAA after making the 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 Craf III were very succulent. They were dehydrated gradually more grades of alcohol to avoid

Corresponding Author: Shahanara Begum, Assistant Professor, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh

more shrinkage (Ali *et al.*, 1999; Haque and Prodhan, 1991; Prodhan and Haque, 1986; Bari and Prodhan, 2001a; Begum and Prodhan, 2003).

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, 2001a; Haque and Prodhan, 1987; Prodhan and Haque, 1986; Sarwar and Prodhan, 2000; Begum and Prodhan, 2003). Serial transverse sections were made at 10-15 μ 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; Hossain *et al.*, 2002; Prodhan and Haque, 1986). Olympus binocular compound microscope (Japan) has been used to investigate the anatomical sections.

RESULTS AND DISCUSSION

Epidermis: The single layer of epidermis in the stem of pigeonpea consists of radially elongated, compact uniform cells as seen in 3-4 days old plants (Fig. 1-5). With the age, the epidermal cells become round, oval or irregular in shape. The epidermis bears multicellular hairs and glandular trichomes (Fig. 1-5). Similar types of hairs are also found in aerial parts of the pigeonpea plants (Bisen and Sheldrake, 1981). After considerable thickening of the abaxial wall a thin layer of cuticle is formed over the epidermis as seen in the basal part of 3-4 days old plant

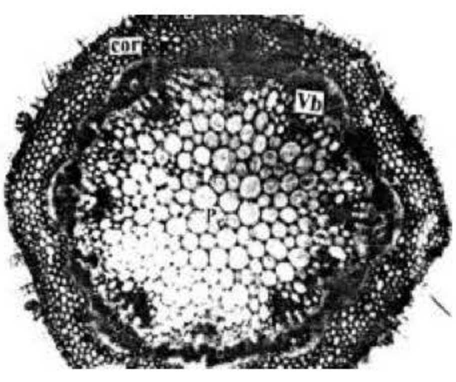

Fig. 1: TS of the apical part of the stem of a 3 days old plant showing epidermis (e) with cuticle, glandular trichomes and hairs, cortex (cor), vascular bundles (Vb) and pith (P) X 260

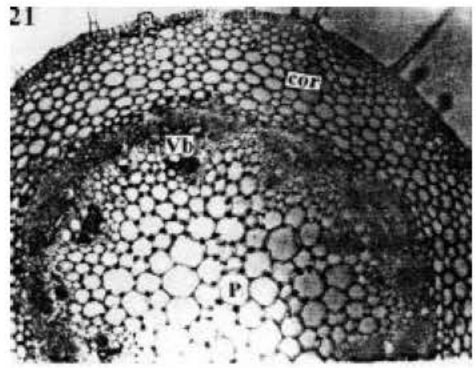

Fig. 2: TS of the middle part of the stem of a 3 days old plant showing epidermis (e) with cuticle, glandular trichomes and hairs, cortex (cor), vascular bundles (Vb) and pith (P)X 260

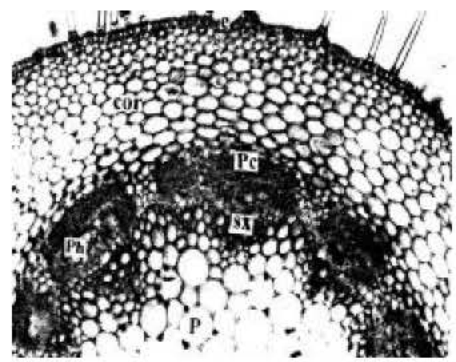

Fig. 3: TS of the basal part of the stem of a 4 days old plant showing epidermis (e) with cuticle, glandular trichomes and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem (Sx), phloem (Ph) and pith (P) X 260

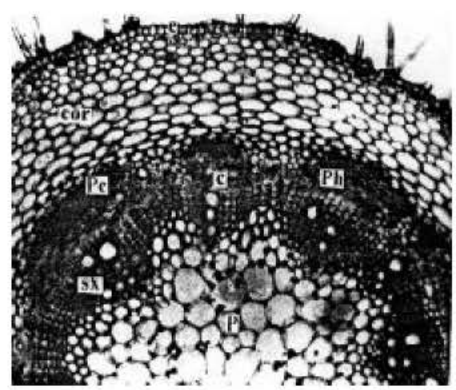

Fig. 4: TS of the basal part of the stem of a 7 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem (Sx), phloem (Ph), cambium (c) and pith (P) X 260

(Fig. 1-3). A thin cuticle has also been reported in the stem of pigeonpea by Hossain (2002). The epidermal cell walls of the stem have been found to be thicker than those of the epidermal cells of root (Begum and Prodhan, 2003). The cell walls of the epidermis may vary in different parts of the plant (Esau, 1965). The cuticle become thickend along with the age of the plant. The cuticularization depends on the type of the plant and the environment (Cutter, 1978; Esau, 1965). The epidermis become disrupted here and there in the mature stem. This is due to the stress of secondary growth and increase in girth.

Cortex: There are 10-14 layers of cortical cells in the stem of pigeonpea (Fig. 1-7). In case of lignosus bean 5-9 layers of cells consists cortex (Bari and Prodhan, 2001a). Some unpublished data shows that, in country bean 5-10 layers (Islam, 2002) and in cowpea 4-7 layers (Begum, 2001) of cortical cells present in the stem. Most of the cells are round, oval or polygonal in shape while others are irregular in shape as seen in 3-4 days old plants (Fig. 1-3). There are small intercellular spaces in cortical parenchyma cells. With the age the stem become tangentially flattened. The number of cortical layers varies according to the age, size and level of secondary growth of the plant.

Primary vascular tissue: The primary vascular tissue appears after the elongation of the first internode of the stem. It forms for a short period as the cambium appears soon. The arrangement of vascular tissues collateral as seen in the stem of 3 days old plant (Fig. 1-2). The vascular bundles are arranged in a ring. There are two types of vascular bundle large and small. One or more small vascular bundles in between two large bundles (Fig. 1-3). The upper part of the stem is not round. It bears small ridges and furrows (Fig. 8). With the age the basal part becomes regular or slightly ridged. The sclerenchymatous cells are present on the abaxial side of the vascular bundles that is pericycle. It is discontinuous in the basal and middle parts of the stem (Fig. 3-7). The pericycle is continuous in the upper part of the stem (Fig. 8). The cells of the pericycle have different origin in different plant (Esau, 1965). So, the origin of this sclerenchymatous tissue of pigeonpea needs a through investigation.

Primary xylem: The large vascular bundle contains xylem and phloem. The primary xylem developes only for a short period. Many vessel members are present in the large vascular bundle (Fig. 2). At the early stage the size of the vessels are more or less similar in size. The vessel members are round, oval or polygonal in shape. All the vessel members are solitary. The cells in between and around the vessel members are mostly axial xylem parenchyma and primary xylem fibres. The primary

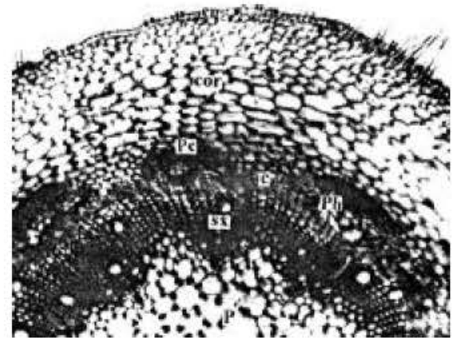

Fig. 5: TS of the basal part of the stem of a 9 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem (Sx), phloem (Ph), cambium (c) and pith (P) X 260

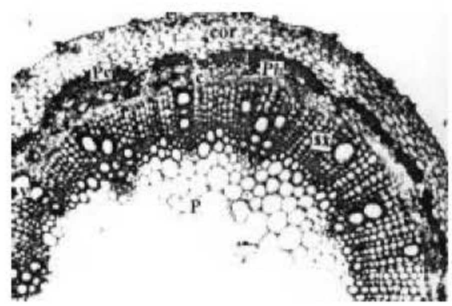

Fig. 6: TS of the middle part of the stem of a 10 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem vessels (V), phloem (Ph), cambium (c) and pith (P) X 260

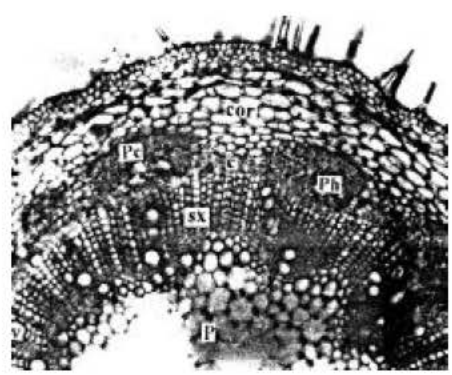

Fig. 7: TS of the basal part of the stem of a 13 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem vessels (V), phloem (Ph), cambium (c) and pith (P) X 260

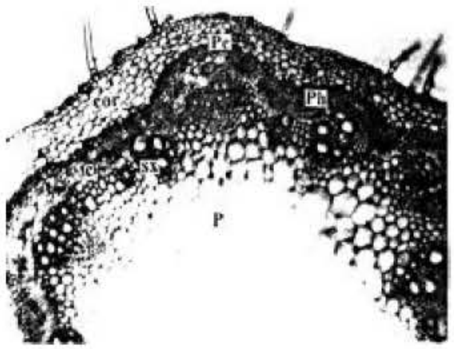

Fig. 8: TS of the apical part of the stem of a 15 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), continuous pericycle (Pc), secondary xylem (Sx), phloem (Ph), cambium (c) and pith (P) X 260

Fig. 9: TS of the basal part of the stem of a 25 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), discontinuous pericycle (Pc), secondary xylem vessels (V), phloem (Ph), cambium (c) and pith (P) X 260

tracheary elements are continued to form in the pigeonpea till the activity of the cambium continues. No secondary growth has been observed in the stem till the plant is 5-6 days old.

Primary phloem: There are several poles of primary phloem abaxial to the primary xylem. In the large bundle there are a number of sieve elements and phloem parenchyma in phloem zone (Fig. 2). The number of phloem poles increases along with the age. Islam (2002), Begum (2001) and Bari and Prodhan (2001a) have reported similar results for country bean, cowpea and lignosus bean, respectively. The primary sieve elements are continued to form in the stem till the activity of cambium continues. No secondary growth has been in the first internode of the stem till the plant becomes 5-6 days old.

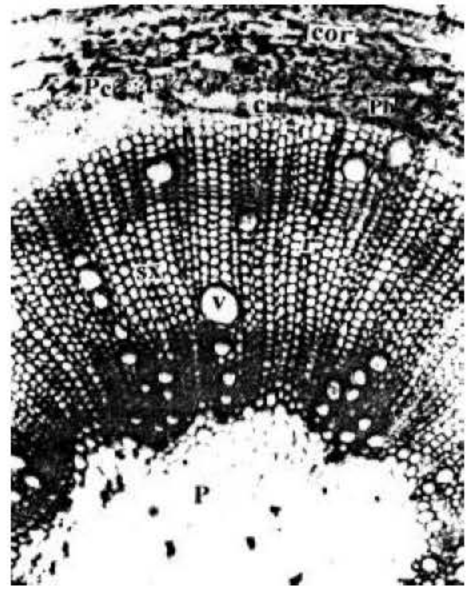

Fig. 10: TS of the basal part of the stem of a 30 days old plant showing epidermis (e) with cuticle and hairs, cortex (cor), pericycle (Pc), secondary xylem (Sx), phloem (Ph), cambium (c), secondary xylem vessel (V), ray parenchyma (r) and pith (P) X 260

Cambium: The cambium has been found to initiate in the primary vascular bundle between xylem and phloem at the basal part of the stem of 3 days old plant. Gradually it extends towards the upper part. The cambial activity of vascular bundle has been observed in the stem of 4-5 days old plant (Fig. 3-5). Soon after the formation of fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially (Fig. 3-7). Similar results have been reported by Haque and Prodhan (1987). After the formation of the cambial ring the cambium becomes active at all points of the ring. In the active state of growth, the cambial zone consists of about 3-5 layers of cells. The cambial cells are tangentially flattened and compact.

Secondary xylem: The secondary xylem has been found in the basal part of the stem after the formation of fascicular cambium in 4-5 days old plant (Fig. 3-4). After a level of secondary growth in the fascicular region, interfascicular cambium is formed and gives rise to the secondary tissues there. After the formation of cambial ring the differentiation of secondary xylem takes place rapidly. The secondary xylem vessels are form on the adaxial side of the cambium (Fig. 3-10). In the mature stem, some vessels are several times larger than the primary xylem vessels. Most of the vessels are solitary and others are paired and multiple as seen in mature stem (Fig. 12). The multiple vessels are composed of 3-4 members. Both large and small solitary vessels are round, oval and polygonal in shape while a few vessels are irregular in shape (Fig. 12). The walls of the vessels are thick and lignified. The spaces between the secondary xylem vessels are filled up with ray, axial parenchyma and fibres. The ray cells are radially elongated. Most of the rays are uniseriate, few are biseriate and some are multiseriate (Fig. 12). The fibre cells are highly lignified, thick walled and small lumen. They are mostly pentagonal, hexagonal or square in shape. The fibre cells remain in between and around the vessels (Fig. 12). The number of layers of secondary xylem increases along with the age. Lots of secondary xylem found in mature stem.

Secondary phloem: The secondary phloem has been found to form abaxially in the fascicular region after the formation of fascicular cambium in 4-5 days old plant (Fig. 3-4). Gradually it extends towards the upper parts of the stem. In the large vascular bundle there are a number of sieve elements and phloem parenchyma in phloem zone. Before maturation of the sieve elements cambium becomes active and gives rise to the secondary phloem. Both primary and secondary sieve elements are well distributed outside the cambial ring. In the mature stem the phloem region increases upto 12-15 layers (Fig. 11). A number of ray and axial parenchyma cells and phloem fibres present in mature stem. Most of the fibre cells are arranged in groups. The groups of fibre cells are arranged in pyramid structure (Fig. 11).

Pith: The pith of the stem of pigeonpea is prominent. The pith is composed of small and large parenchymatous cells (Fig. 1-10). The pith cells are round, oval, pentagonal, hexagonal or somewhat polygonal in shape. The pith increases in size due to increases in diameter of the pith cells as well as the size of the intercellular spaces. Due to stress of axial growth of the stem and the addition of secondary xylem towards the center, the peripheral pith cells lose their intercellular spaces and become narrow (Esau, 1965).

Periderm: The well developed periderm has been observed in basal part of the stem of pigeonpea (Fig. 11). 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 mature stem. The number of cork cells in a radial rows depends on the age and size of the plant (Cutter, 1978; Esau, 1965). In *Sesbania* of papilionaceae, a well developed periderm has been reported by Sarkar (1996, unpublished data). The development and morphology of different components of periderm have not been studied during the present investigation.

Fig. 11: TS of the stem of a mature plant showing periderm (Pr), secondary phloem (Sp), secondary phloem fibres (F), secondary xylem (Sx), cambium (c), secondary xylem vessel (V) X 260

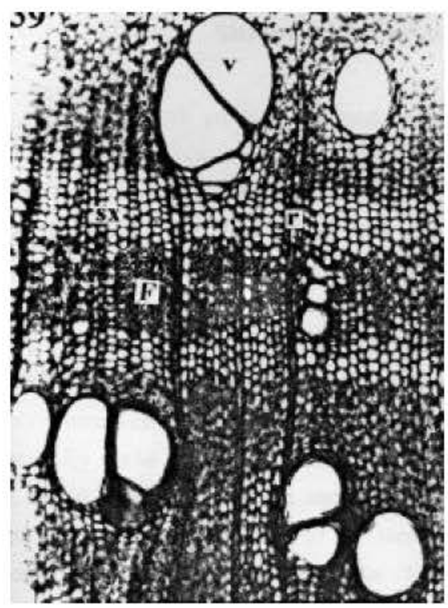

Fig. 12: TS of the stem of a mature plant showing secondary xylem (Sx), secondary xylem vessels (V), ray parenchyma (r), xylem fibres (F) and axial parenchyma X 260

CONCLUSIONS

The primary vascular tissue appears after the elongation of the first internode of the stem of pigeonpea. The internode between cotyledonary node and first leaf is considered as the first internode of the stem. The vascular bundles are collateral and arranged in a ring. The sclerenchymatous cells constitute the pericycle are present on the abaxial side of the vascular bundles. The pericycle is discontinuous in the basal part of the stem. The epidermis is single layered. The epidermis ruptures here and there and the cells become partly or wholly disorganized due to the stress of secondary growth. The cambium initiates in primary vascular bundle between xylem and phloem of 3 days old plant. The activity of cambium is similar to the woody dicityledonous herb. After the formation of fascicular cambium it gives rise to secondary xylem adaxially and secondary phloem abaxially. After the disintegration of the epidermis, the phellogen appears in the cortex and gives rise to cork cells abaxially and phelloderm adaxially. The pith resembles a typical dicotyledonous stem.

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