

## Botanical Studies On *Phaseolus Vulgaris* L. II-Anatomy Of Vegetative And Reproductive Organs

<sup>1</sup> Rania M. A.Nassar, <sup>2</sup> Mohamed S. Boghdady and <sup>3</sup> Yasser M. Ahmed

1- Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

2- Department of Agricultural Botany and Plant Pathology, Faculty of Agriculture, Zagazig University, Egypt.

3- Department of Vegetable Crops, Faculty of Agriculture, Cairo University, Giza, Egypt.

[ranianassar23@yahoo.com](mailto:ranianassar23@yahoo.com)

**Abstract:** The present study is concerned with the histological features of Kidney bean plant. The anatomical structure of different vegetative and reproductive organs was investigated fortnightly throughout the whole growing season. Studied organs included main root, main stem (represented by apical and median internodes), different types of foliage leaves developed on the main stem and on lateral shoot; including lamina and petiole, flower bud, fruit and seed. Histological features of various organs of Kidney bean plant were analyzed microscopically and photomicrographed. [Journal of American Science. 2010;6(12):217-229]. (ISSN: 1545-1003).

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### 1. Introduction

In the first part of this study (Nassar *et al.*, 2010), the authors investigated the morphology of vegetative and reproductive growth of Kidney bean plant (*Phaseolus vulgaris* L.) throughout the consecutive stages of its whole life span. Consequently, in this part of the study, it is aimed to bring to light more information about the anatomical structure of vegetative and reproductive organs of the plant during successive ages of its whole life span in order to complement the phytophany study of Kidney bean plant which started in the first part.

Obviously, continued acquisition of new information about different botanical aspects of this species, which is of great interest from the economic point of view, are required.

### 2. Materials and Methods

The present study was carried out to investigate the anatomical structure of vegetative and reproductive organs of Kidney bean plant (*Phaseolus vulgaris* L.) of the family Fabaceae.

Therefore, a field trial was conducted in the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the summer growing season of 2009 to provide the experimental plant materials. The work of microtechnique was carried out at the Laboratory of the Agricultural Botany Department, Faculty of Agriculture, Cairo University during the period from May, 2009 till April, 2010.

The field trial included five replicates, each represented by one plot. The plot was 4×5 m with eight ridges 60 cm apart. Date of cultivation was May 12<sup>th</sup>, 2009. Seeds of Kidney bean plant cv. Giza 6 were sown in hills spaced 25 cm on one side of the ridge. The plants were thinned to two plants per hill. All field practices were carried out as recommended for the studied crop in the vicinity.

A full microscopical study was carried out to investigate the histology of Kidney bean plant. Samples representing different plant organs were taken periodically, fortnightly, throughout the growing season. The following were investigated:

1. The main root, 1 cm below the hypocotyl.
2. The main stem represented by terminal and median internodes.
3. The mature prophyll and the apical leaflet of the foliage compound leaves number 3, 6 and 9 on the main stem, and of compound leaves representing secondary branches. The petiole of the compound leaf.
4. Flower bud.
5. Mature fruit and seed.
6. Type of stomata was defined using epidermal peals.

Microtechnique procedures given by Nassar and El-Sahhar (1998) were followed. Specimens were killed and fixed for at least 48 hrs in FAA solution, (10 ml formalin, 5 ml glacial acetic acid and

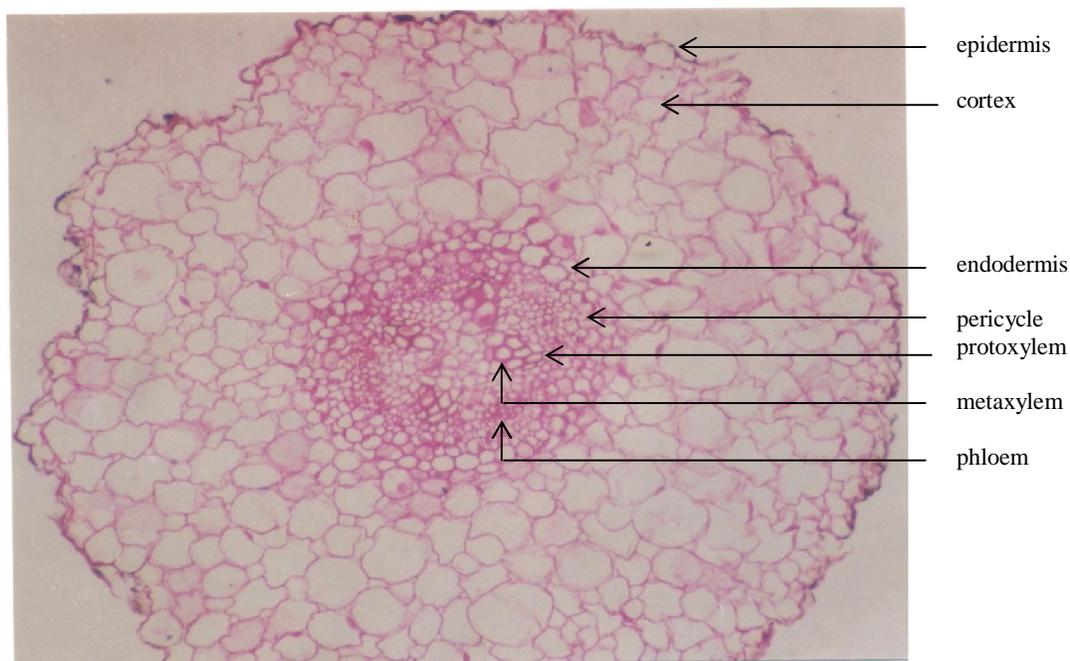
85 ml ethyl alcohol 70%), washed in 50% ethyl alcohol and dehydrated in a series of n-butyl alcohol before embedded in paraffin wax (mp 56-58°C). Transverse sections which were cut on a rotary microtome to a thickness of 20 microns were stained with crystal violet/erythrosin before mounting in Canada balsam. Slides were analyzed microscopically and photomicrographed.

### 3. Results and Discussion

#### 1- Structure of the main root:

The anatomical structure of Kidney bean root system was investigated through transverse sections of the main root at different stages of plant growth. The tap root of two weeks old seedling, as shown in transverse section (Figure 1), is in primary state of growth. It has an uniseriate epidermis of tabular shaped cells. Cuticle on the outer walls and stomata are absent. Some epidermal cells prolong to form the typically unicellular root hairs. The cortex composed of about 6 layers of thin-walled irregular

parenchyma cells with well-developed intercellular space system. The innermost layer of the cortex is the endodermis, an uniseriate zone of small barrel-shaped cells forming a distinct layer surrounding the stele. Next to the endodermis lies a layer of thin-walled parenchyma cells forming the pericycle. The vascular bundle is radial. Xylem and phloem occur in separate patches arranged on alternate radii, intervened by small parenchyma cells. The latter forms the conjunctive tissue. The bundle is tetrarch since four patches of xylem alternate with equal number of phloem patches. Protoxylem vessels occur towards the periphery and metaxylem towards the center, thus showing centripetal mode of differentiation from the procambium. This is the typical exarch xylem of roots. Opposite the protoxylem, pericycle is made up of two or three layers showing the point of origin of lateral roots. The center portion of the stele is occupied by a few compactly arranged thin-walled paranchymatous cells without any inter-cellular spaces forming a very small region of pith.



**Fig.(1):** Transverse section through the main root of *Phaseolus vulgaris* L. plant, aged two weeks, showing its primary structure. (X 144)

At the age of four weeks (Figure 2), the main root is in secondary state of growth, the epidermis as well as the cortex are completely sloughed off and a continuous well defined periderm arising in the pericycle is present. A cambial zone of about four layers is seen and secondary thickening proceeds, the xylem being more in amount than the phloem. The formation of parenchymatous rays from the cambium which was originated in the pericycle opposite to the xylem ridges is clearly obvious in this stage of secondary growth.

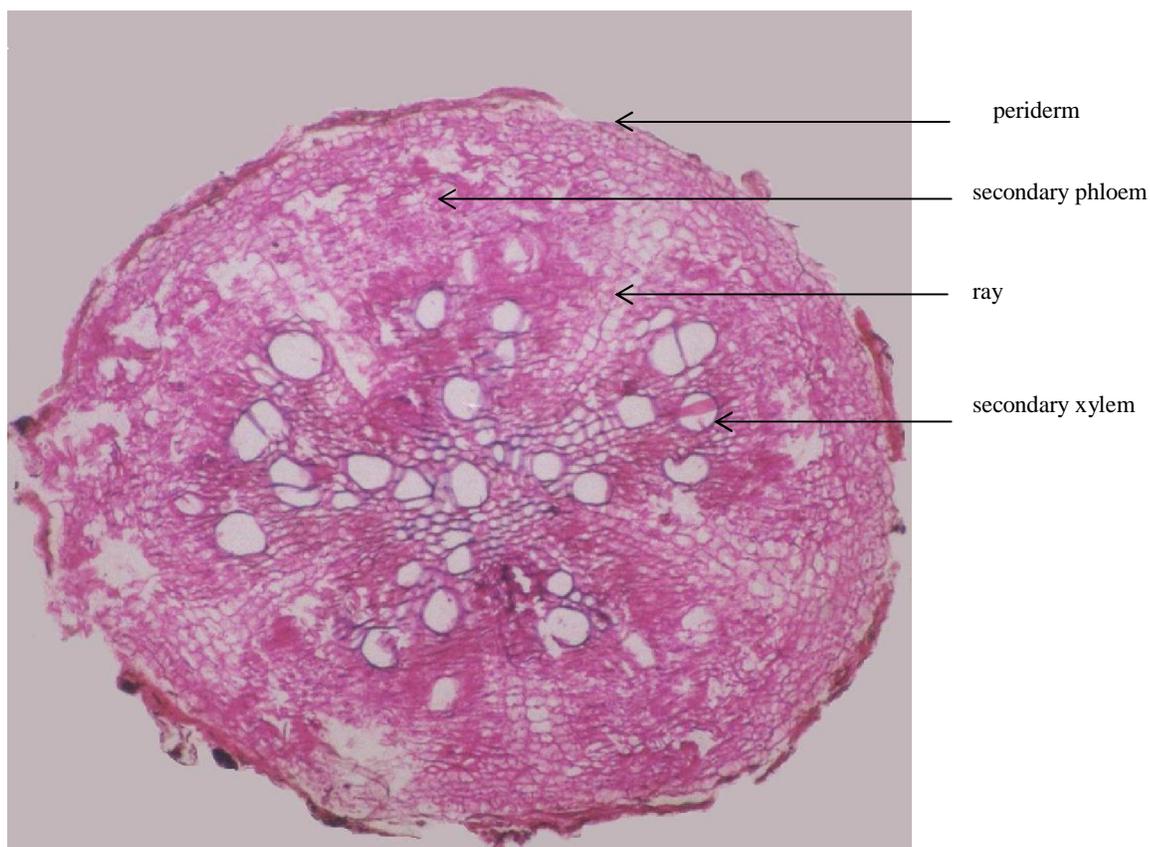
The secondary thickening is more prominent as tested plants were six weeks old (Figure 3). At this age, the root comprises mainly of a vascular cylinder surrounded by a periderm. The secondary xylem contains vessels of various diameters which accompanied by fibers and abundant amount of parenchyma cells.

As far as the authors are aware, no detailed study dealing with the anatomical structure of Kidney bean roots was carried out.

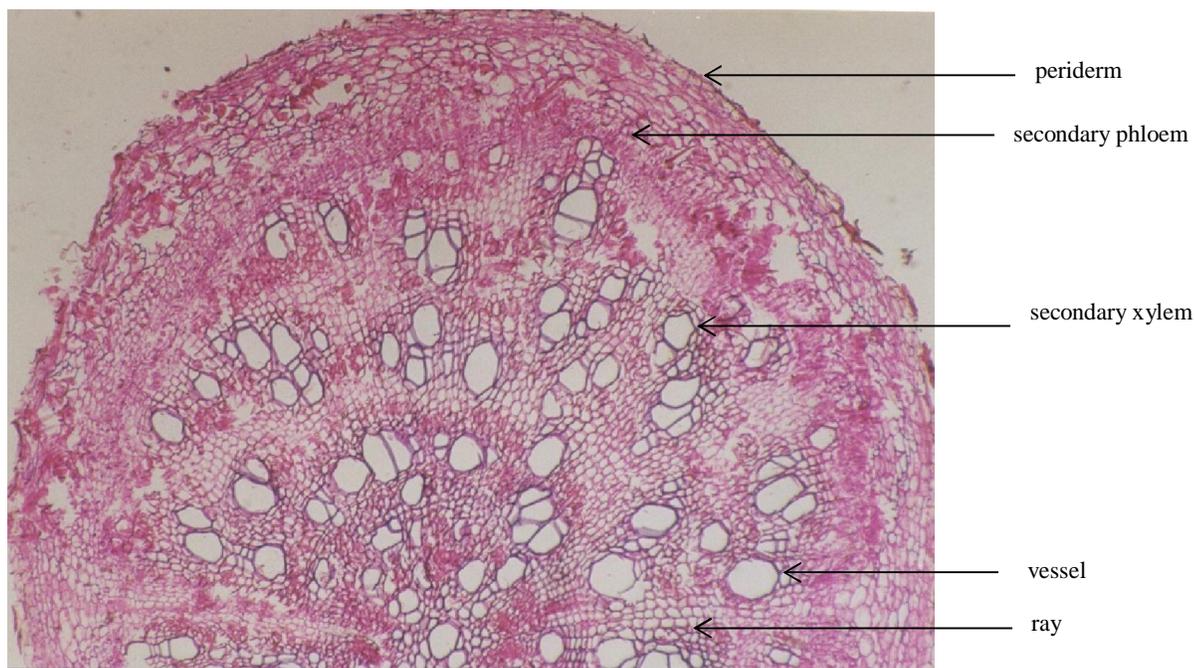
## 2- Structure of the main stem:

### a- The apical internode:

The apical internode of the main stem was studied from the anatomical point of view at the age of six weeks to disclose the primary structure of the main stem. The transverse section shown in Figure (4) reveals that the stem surface of Kidney bean plant directly below the shoot apex is strongly ridged and fluted, almost pentagonal in outline. The epidermal cells are nearly square in shape and covered with a thin layer of cuticle. Stomata of paracytic type are present at the same level of the epidermis, each consisting of two guard cells and two accessory ones. Trichomes of nonglandular hairs are observed. The ridges of the stem mainly consist of collenchyma. The cortex at the furrows between the ridges is composed of one or sometimes two layers of collenchyma cells underlying the epidermis followed often by two layers of chlorenchyma and four layers of thin-walled parenchyma cells. A few layers of sclerenchyma forming a nearly continuous band occur next to the last layer of the cortex, it may be called pericycle or perivascular tissue.



**Fig.(2):** Transverse section of the main root of *Phaseolus vulgaris* L. plant, aged four weeks, showing its secondary structure. (X 52)



**Fig.(3):** Transverse section of the main root of *Phaseolus vulgaris* L. plant, aged six weeks, showing an advanced stage of secondary growth. (X 52)

The vascular bundles are almost arranged in a ring, being separated from each other by wide panels of parenchyma tissue which are a part of the ground tissue. There are five major collateral bundles located opposite to the corners. In addition, there are two to four minor collateral bundles between any of two major ones lying opposite to the furrows. The major bundle has 13 to 18 vessels in nearly parallel rows. The minor bundle often comprised of one row contains 3 to 6 vessels.

The pith, which comprises a large portion of the stem core, consists of thin-walled polygonal parenchyma cells which tend to decrease in size towards the periphery.

#### **b- The median internode:**

The transverse section through the median internode of the main stem of Kidney bean plant at the age of four weeks is shown in Figure (5). It is obvious that the main stem at its median portion is often ribbed; *i.e.*, polygonal in outline. Worthy to note that the ribs are comparatively smaller in size than those associated with the apical internode and the secondary growth takes place in nearly a continuous cylindrical form.

The epidermis which still having intact cells shows active dilation accompanied by radial division to accommodate with the increase in stem circumference. Also, many of both kinds of cortical cells (collenchyma and parenchyma) show elongation in the tangential direction accompanied sometimes by radial divisions. Stomata are present at the same level of the epidermis. Trichomes are not observed. The cortex composed of about seven layers of which the outer two layers are collenchyma underlying the epidermis and the five rest layers are parenchyma. The innermost layer of the cortex, the starch sheath, is easily recognized. The pericyclic cells show their transformation into fibers abutting on the collateral bundles of the stele. Thus, an incomplete ring of fibrous strands are developed.

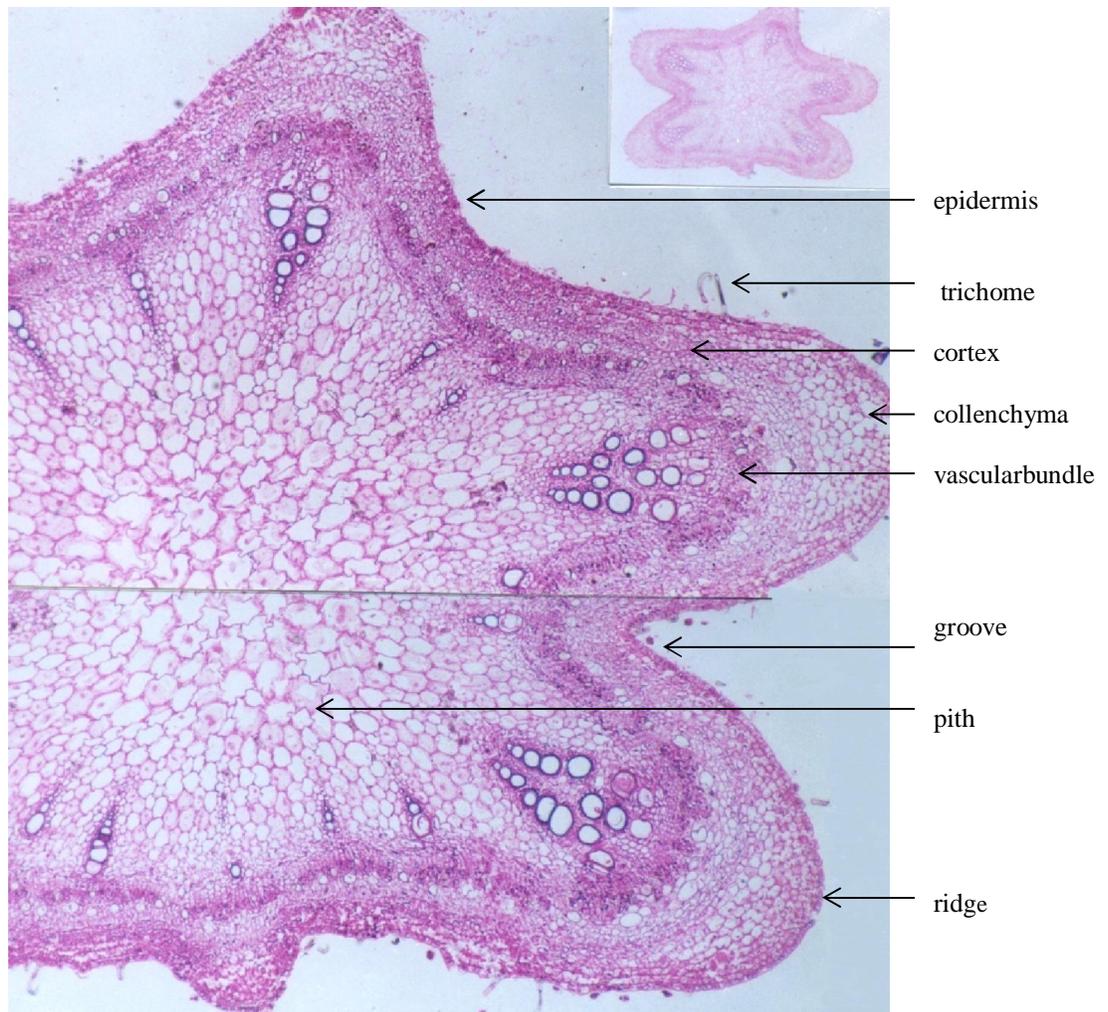
The stele consists of 21 collateral bundles arranged in a ring, being separated from one another by two to three rows of well lignified parenchyma cells. The bundles are relatively different in size. There are seven large bundles located opposite to the ridges which being seven in number. There are two intermediate bundles between any of two large ones lying opposite to the furrows. Secondary thickening proceeds and secondary growth takes place in nearly a continuous cylindrical form. The secondary phloem

increases considerably in amount. The secondary xylem has an increased amount of vessels present in nearly radial rows, and the ground tissue where the vessels are embedded is formed of lignified parenchyma cells. The large bundle has 48 to 52 vessels, while the intermediate bundle has 33 to 38 vessels. A complete cambial ring is formed by the continuity of the interfascicular cambium with the fascicular one. The primary xylem is recognized abutting on the pith.

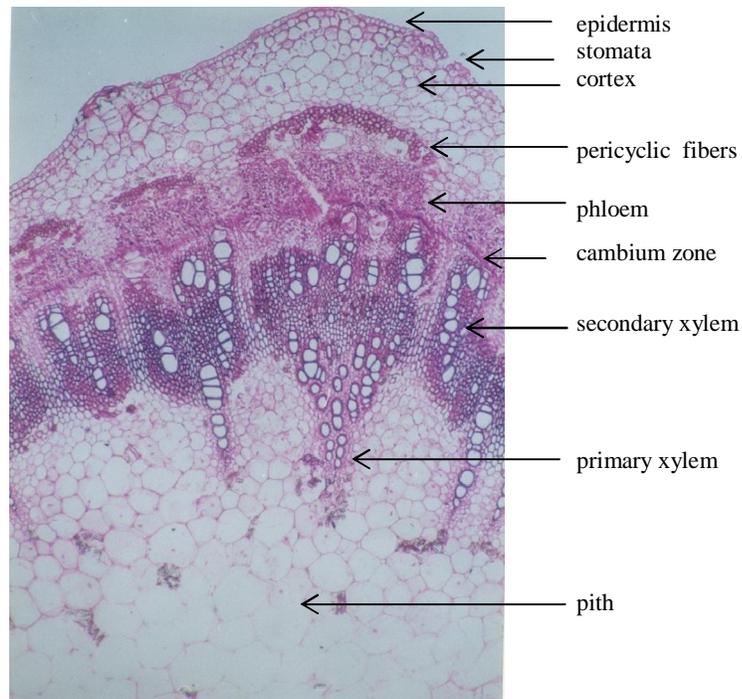
The pith consists of polygonal parenchyma cells which tend to decrease in size towards the periphery. Small triangular intercellular spaces are visible.

At the age of 10 weeks, secondary thickening reached its maximum and the stem surface being cylindrical in outline (Figure, 6). The xylem vessels are mostly arranged in radial rows embedded in well lignified parenchyma cells. The vessels are present solitary or in radial groups, each consists of 2-4 vessels. Vascular rays mostly of 2 cells wide are observed and the pith at the center of the section being hollow (destroid).

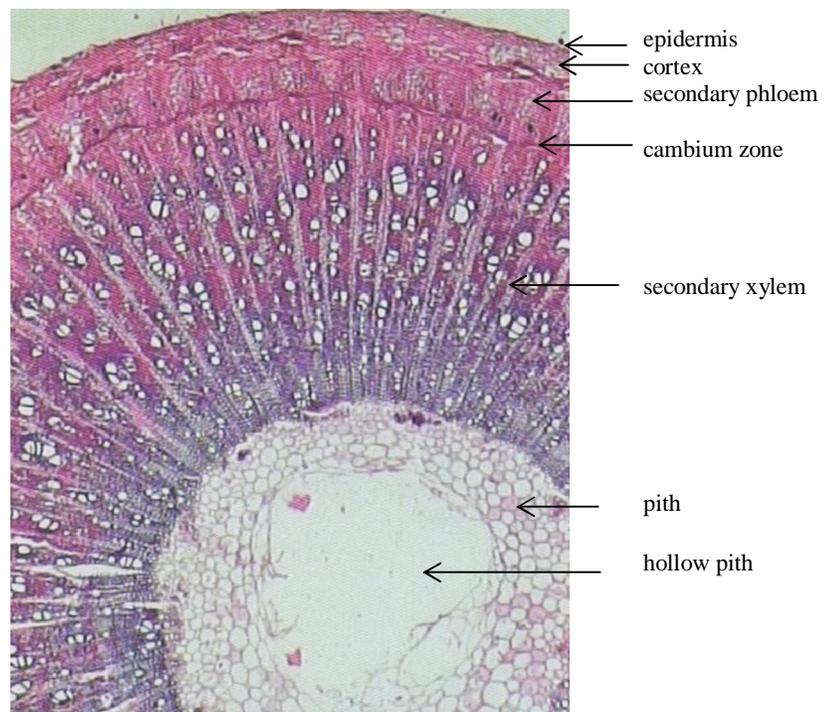
At the extent of the authors knowledge no detailed studies dealing with the anatomical structure of Kidney bean stem are available.



**Fig.(4):** Transverse section through the apical internode of the main stem of *Phaseolus vulgaris* L. plant at the age of six weeks. (X 52)



**Fig.(5):** Transverse section through the median internode of the main stem of *Phaseolus vulgaris* L. plant at the age of four weeks. ( X 52 )



**Fig.(6):** Transverse section through the median internode of the main stem of *Phaseolus vulgaris* L. plant at the age of ten weeks. ( X 52 )

### 3. Structure of the leaf:

#### a- Leaf blade (lamina):

From the morphological point of view, Kidney bean plant develops two types of leaves as follows:

- i- Two simple basal opposite prophylls.
- ii- Pinnately trifoliate compound leaves which are alternately arranged on the main stem and the lateral branches.

Therefore, the anatomical structure of leaf blades representing these two different types of leaves were investigated. Transverse sections of mature prophyll as well as of apical leaflet of the compound leaf number 3, 6 and 9 on the main stem and of compound leaves on lateral branches were examined.

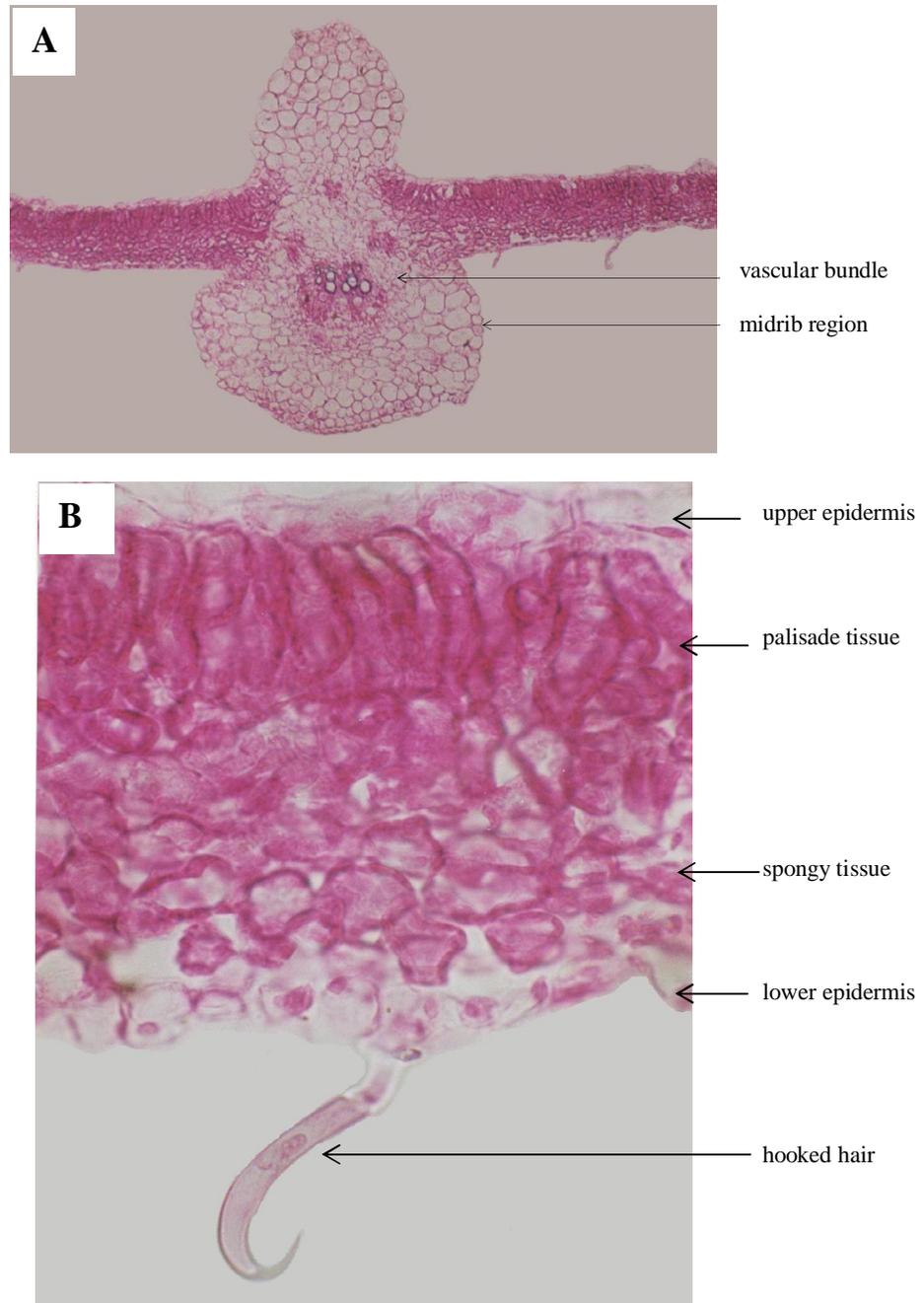
It was found that both types of leaves developed by Kidney bean plant almost have the same structure. Generally, they are dorsiventral and composed of three tissue systems as follows:

- i- Epidermal tissue system consists of the epidermal layers occurring on the adaxial (upper) and the abaxial (lower) sides.
- ii- The ground tissue system, which is known as the mesophyll tissue, is always differentiated into columnar palisade parenchyma on the adaxial side and irregular or isodiametric spongy parenchyma on the abaxial side and this means that the leaves are dorsiventral.
- iii- The vascular tissue system is composed of vascular bundles which are usually collateral and form the skeleton of the leaf on which other tissues, the ground tissue, remain inserted. At the midrib, the principle vascular vein develops. In addition, smaller lateral ones constitute the reticulate system of venation.

Transverse section through the median portion of the mature prophyll of Kidney bean plant, two weeks old, (Figure 7) reveals that it consists of two epidermal layers with a mesophyll in between. Both the epidermal layers are uniseriate, composed of nearly compactly arranged rectangular cells with thin rounded cuticularised outer walls. Stomata occur on both sides, being more frequently present on the lower epidermis. Likewise, trichomes of nonglandular hairs, mostly hooked hairs with short basal cells and a large bent terminal cell are present on both surfaces, being more frequently present on the lower epidermis (Figure 7). The mesophyll is differentiated into palisade and spongy cells. The palisade tissue consists of one layer of cells elongate perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies of the whole thickness of the mesophyll. The spongy tissue occurs towards the

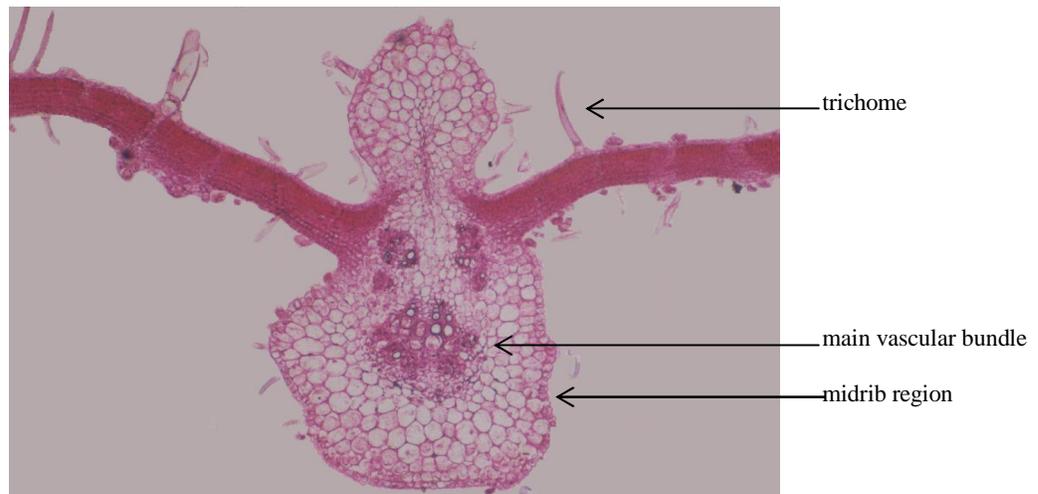
lower epidermis and consists of 4 to 5 layers of chlorenchymatous loosely arranged cells with relatively wide intercellular spaces. At the midrib region, both upper and lower epidermis are convex. The bundle in the midrib is the largest in the prophyll and the lateral ones decrease in size towards the margins. The vascular bundle is oriented with the xylem directed toward the adaxial surface and the phloem toward the abaxial one. The vascular bundle of the midvein is embedded in a ground tissue of parenchyma cells with a mass of collenchyma cells underlying the two epidermis. Whereas, the smaller bundles are directly embedded in the mesophyll.

The anatomical structure of leaf blades representing the mature foliage compound leaf of Kidney bean at different stages of plant growth was also investigated. It was found that all investigated leaves have, in general, the same structure. Leaflets are dorsiventral; *i.e.*, the palisade tissue is located on the adaxial side of the blade and the spongy tissue on the abaxial one (Figures 8 and 9). There are two epidermal layers on adaxial and abaxial surfaces of the leaflet. Each is uniseriate, composed of a row of compactly-set tabular cells. The outer walls are cutinised and possess thin cuticle. Stomata occur on both surfaces, being more numerous on the lower epidermis than on the upper one. They are of rubiaceous or paracytic type (Figure 10). The stoma consisting of two guard cells and two accessory ones, subsidiary cells flank the stoma parallel with the long axis of the guard cells. At the midrib region, both the upper and lower epidermis are convex. Trichomes are present on both surfaces, being more numerous on the upper epidermis than on the lower one. They are nonglandular uniseriate with short basal cells accompanied by an elongated terminal cell and most of them being hooked with a large bent terminal cell. The palisade tissue consists of two layers of slender cells of dense plastids, occupying almost one-half of the whole thickness of the mesophyll. The spongy tissue is composed also of 2 to 3 layers of chlorenchymatous cells. There is a mass of collenchymatous cells below the adaxial and abaxial epidermis at the midrib region. Therefore, the included bundle, the principle one, is not directly embedded in the mesophyll as do the smaller ones. The midrib bundle consists of a larger strand opposite to two smaller ones. The midrib is also supported by a fibrous strand, a crescent like cap, abutting on the phloem of its large collateral bundle.

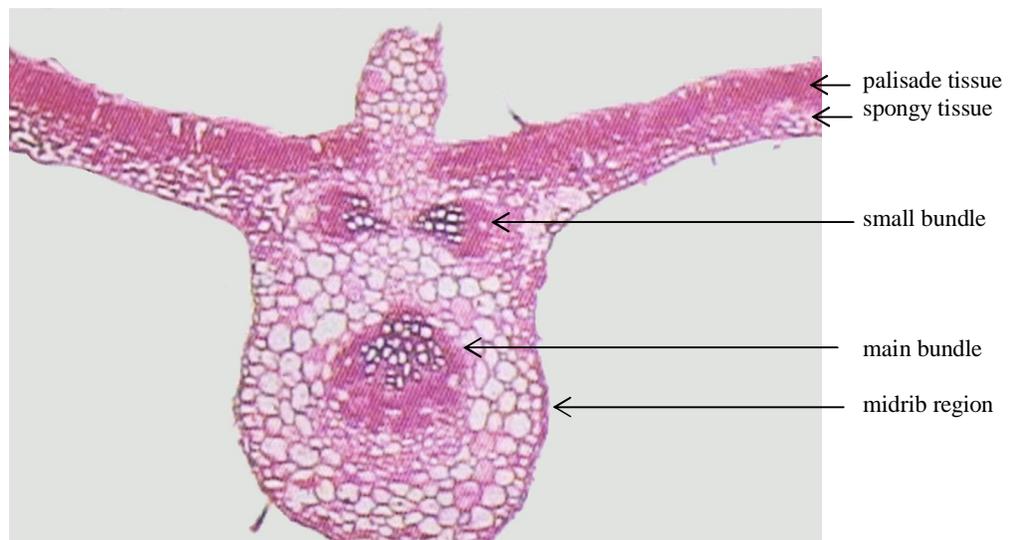


**Fig.(7):** Transverse section through mature prophyll of *Phaseolus vulgaris* L. plant, two weeks old.

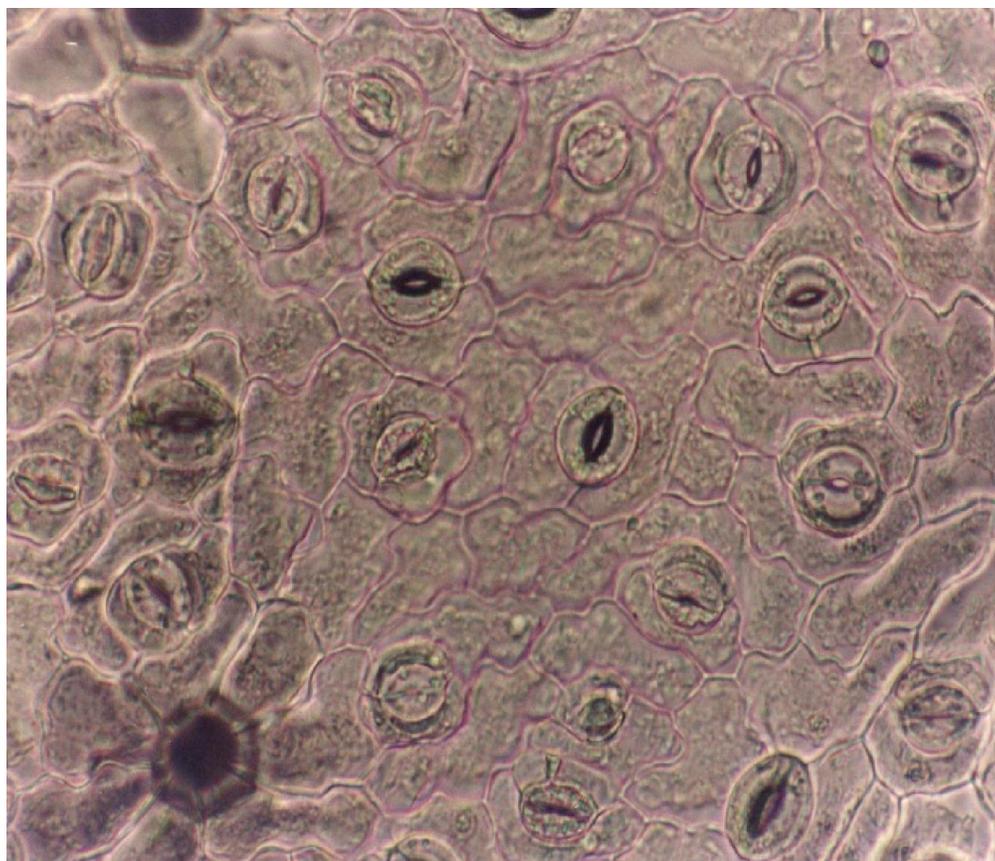
- A- Whole section. ( X 52 )  
B- Magnified portion of A. ( X 540 )



**Fig.(8):** Transverse section through the apical leaflet of the third foliage compound leaf on the main stem of *Phaseolus vulgaris* L. plant at the age of four weeks. (X 52)



**Fig.(9):** Transverse section through the apical leaflet of a foliage compound leaf developed on the fourth lateral branch of *Phaseolus vulgaris* L. plant at the age of eight weeks. ( X 52 )



**Fig.(10):** Epidermal peel showing the paracytic stomata type in leaflet of *Phaseolus vulgaris* L. plant. ( X 144 )

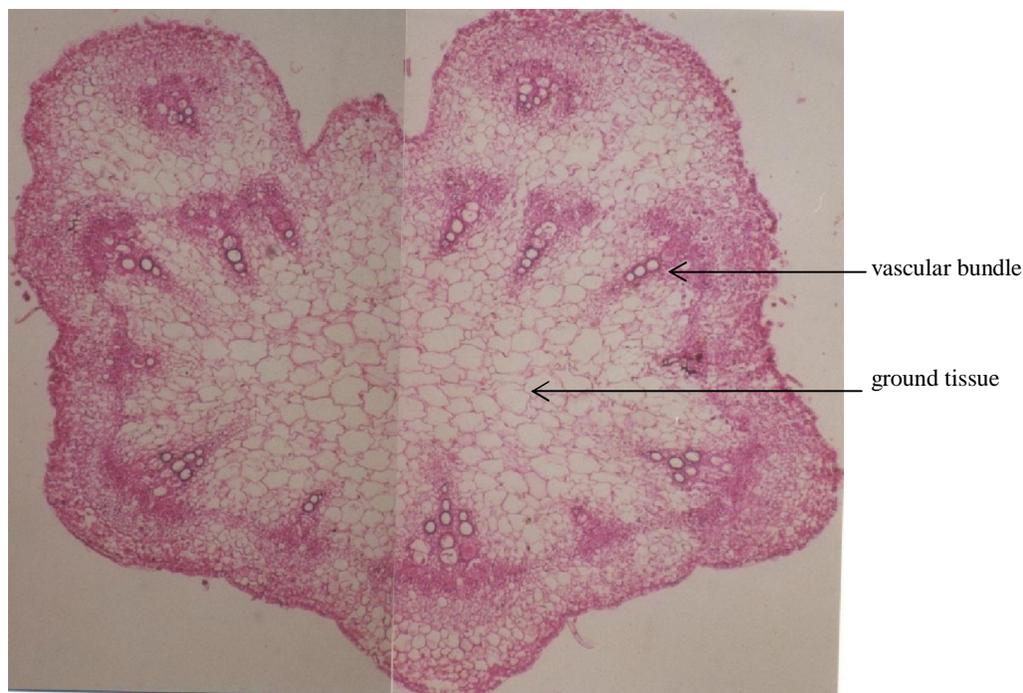
In this respect, Metcalfe and Chalk (1979) recorded non-glandular hairs in the genus *Phaseolus*. Uniseriate, with a variable number of short basal cells, accompanied by an elongated terminal cell. Hooked hairs, with short basal cells, and a large bent, terminal cell.

**b- Leaf petiole:**

The petiole of Kidney bean leaf as seen in transverse section (Figure 11) is polygonal in outline (almost pentagonal) with two lateral wings at the corners of the adaxial side. The petiole is bounded by an uniseriate epidermis of nearly square shaped cells. The outer walls of the epidermis are somewhat thickened and covered with a thin layer of cuticle.

Stomata and trichomes, similar to those found on the main stem and leaf blades, are present. The ground tissue consists mostly of relatively large parenchyma cells. The angles, beneath the epidermis, consists mainly of collenchyma cells. Inside the collenchyma there is a zone of chlorenchymatous cells ranging from 2 to 3 layers ending at the vascular cylinder. The chlorenchyma cells decidedly smaller than the residual parenchyma cells of the ground tissue.

The vascular tissues are formed of 13 collateral bundles arranged in a ring plus the two accessory small bundles in the ground tissue of the two lateral wings. The bundles are separated from one another by wide areas of ground tissue. The xylem vessels are arranged in radial rows, being in most bundles of one row.



**Fig.(11):** Transverse section through the petiole of the third foliage compound leaf on the main stem of *Phaseolus vulgaris* L. plant at the age of four weeks. (X 52)

Worthy to note that there are small groups of incompletely differentiated phloem between the bundles and ill differentiated fibrous cap abutting on the phloem of each collateral bundle was observed.

#### 4- Structure of the floral bud:

A transverse section through the floral bud of Kidney bean plant is shown in Figure (12). It is clear that the sepals of the calyx are united and comprised of two epidermal layers and 4-5 layers of ground tissue in between. There are numerous traces which extending through the ground tissue. The corolla is papilionaceous with one posterior petal (the standard), two lateral petals (the wings) and two lower united anterior petals (the keel). Each segment consists of two epidermal layers of nearly square-shaped cells surrounding 2-6 layers of parenchymatous cells forming the mesophyll. Many traces are extending through the mesophyll.

The stamens are ten; each consists of a two-lobed tetrasporangiate anther borne on the filament, a thin stalk with a single vascular bundle. The androecium is diadelphous, since the posterior stamen is free and the other nine stamens are with united filaments from the base to nearly more than half of their length while the anthers are free. The stamens form an open tube enclosing the long ovary. The upper parts of the filaments bend toward the banner.

The gynoecium is composed of a single carpel and the ovary is of one locule. Placentation is marginal. The ovary contains two bundles at the placenta side (the dorsal bundles) and a single one at the opposite side (the ventral bundle).

#### 5- Structure of the fruit and the seed:

The fruit of Kidney bean is a simple dehiscent legume which develop from a single carpel, cylindrical, constricted between the seeds, splitting along both sutures at maturity. The legume has two lines of dehiscence; one through the union of the carpel margin and the other along the median vascular bundle.

Transverse section through the mature fruit of Kidney bean is shown in Figure (13). The pericarp, the wall of the matured ovary, consists of three distinct layers; namely, the exocarp, the mesocarp and the endocarp. The exocarp includes the epidermis and a subepidermal layer of 1-2 cells in thickness, both are composed of thick-walled cells. The mesocarp consists of several layers lying next to the exocarp. The component parenchymatous cells of these layers are characterized by their rather thick and slightly lignified walls.

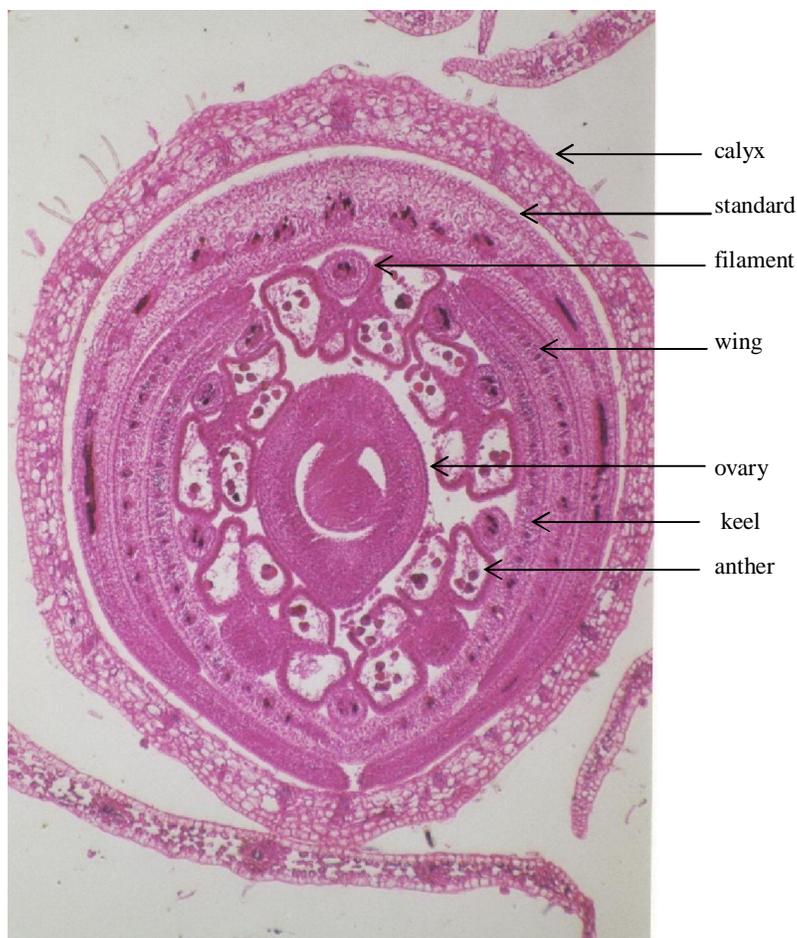
They are tangentially elongated and deeply stained. The mesocarp contains the vascular traces supplying the pod. The endocarp comprises the

remainder of the parenchymatous cells of the fruit wall, the pericarp, and the inner epidermis. The parenchymatous cells are weakly stained being thin-walled and much enlarged in different planes. The inner epidermis though still intact, its constituent cells become weakly outlined and thus could be hardly described.

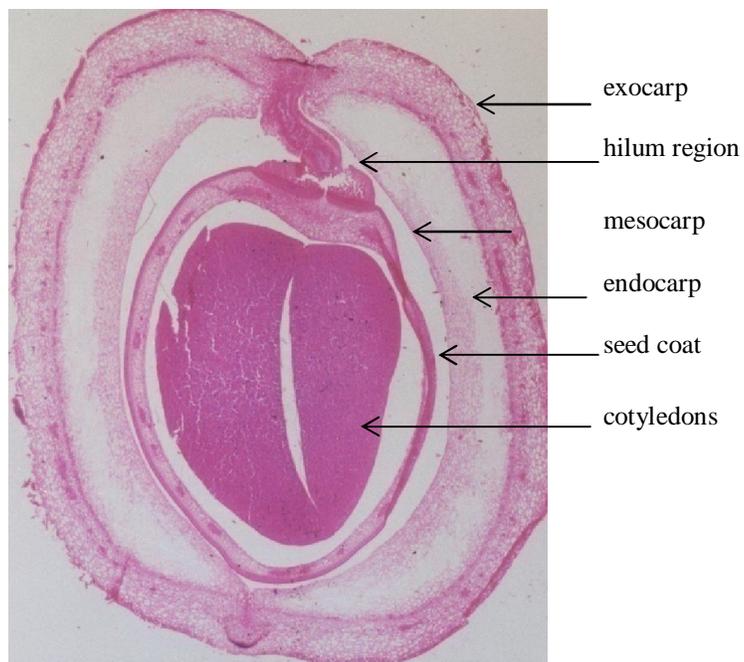
The seed coat, the integuments of mature ovule, differentiates into a variety of distinct layers. The outermost layer, the epidermis remains uniseriate and develops into the palisade layer characteristic of leguminous seeds. It is composed of macrosclereids with unevenly thickened walls. The cells of the subepidermal layer differentiate into the so-called columnar cells, also termed pillar cells or hourglass cells. The layers beneath the subepidermal layer are of bigger and tangentially elongated parenchyma cells with the innermost layers being largely pressed.

The vascular system is well developed, it is an extension of the vascular bundle from the funiculus to the chalazal region where it branches. Finally, the inner epidermis characterized by very poorly outlined cells, and with rather thick lignified walls. It is worthy to note that, two palisade layers occur in the hilum region. The outer of these is derived from the funiculus and the inner belongs to the seed coat. Moreover, a compact group of cells of unknown role occurs in the hilum region. They are referred to by Esau (1959) to be tracheids. The seed coat envelops the embryo which consists of two large cotyledons, plumule, and the radicle. The cotyledons have big thin-walled cells, rich in starch grains of conspicuously large size.

The above description of the seed coat is in agreement with that given by Esau (1959).



**Fig.(12):** Transverse section of the floral bud of *Phaseolus vulgaris* L. plant. ( X 52 )



**Fig.(13):** Transverse section of the mature pod of *Phaseolus vulgaris*L. plant, showing the structure of the fruit and the seed. ( X 12 )

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