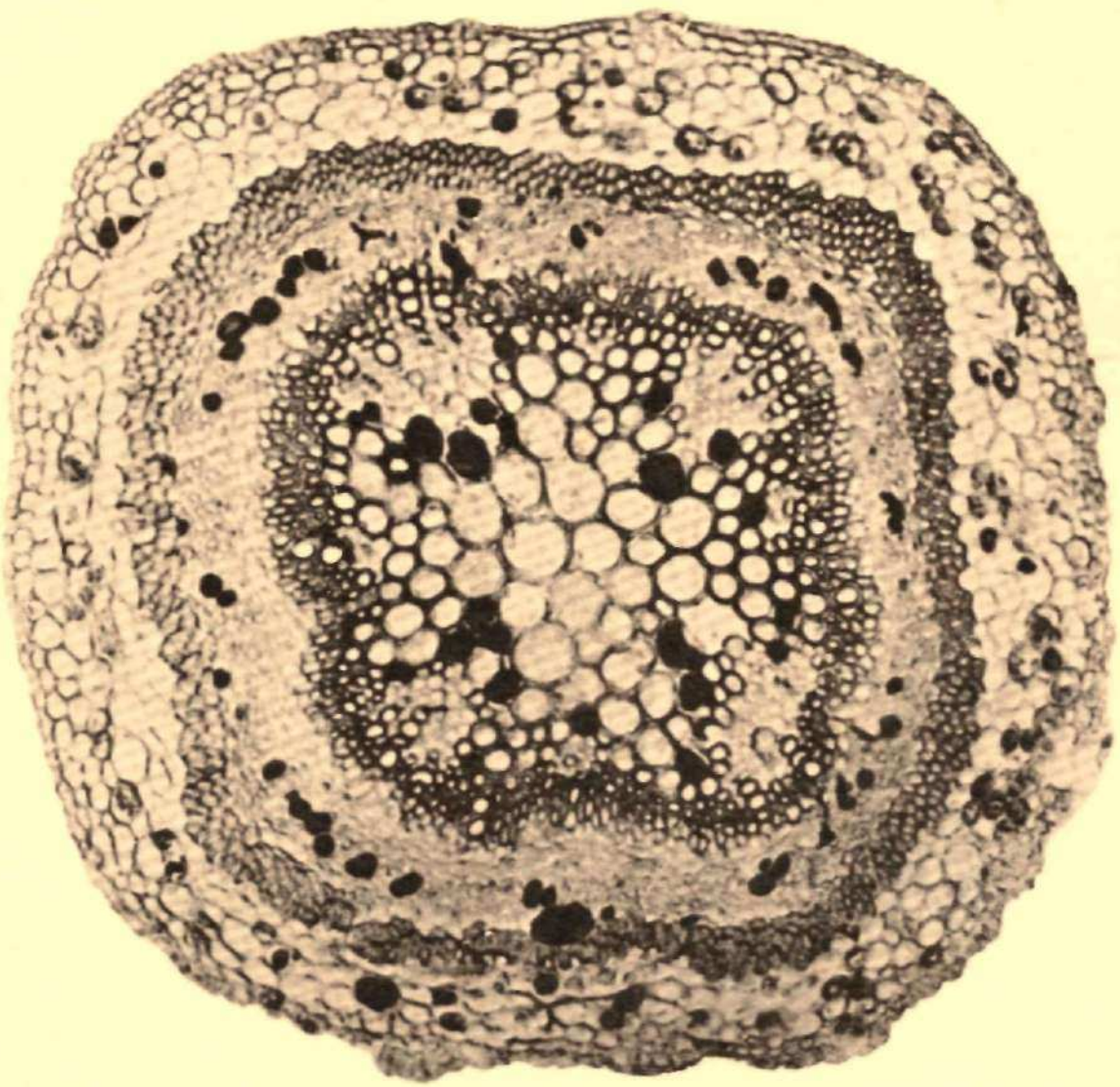
THE ANATOMY OF THE PIGEONPEA



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The Anatomy of the Pigeonpea

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CONTENTS

Introduction

During the 3 years 1974-77 we studied the anatomy of most of the tissues and organs of the pigeonpea and, in the course of this work, have built up a collection of permanent microscope slides. These are retained in the Anatomy Laboratory at ICRISAT as a reference collection and may be consulted by anyone who is interested.

This report contains a brief and preliminary description of pigeonpea anatomy. We have studied the anatomy of several different cultivars; unless otherwise indicated, the following general descriptions apply to all cultivars investigated. We have not noticed any striking qualitative anatomical differences among cultivars; no doubt quantitative differences exist, but these are difficult to establish with anatomical methods involving very small samples.

Many of the features of the anatomy of the pigeonpea are similar to those of other dicotyledonous plants, described in standard textbooks of anatomy. We have not attempted to duplicate these descriptions. Some aspects of the anatomy of the pigeonpea have been covered in detail by Dr. P. Venkateshwara Rao in his Ph.D. thesis under reference (no date). A copy of this thesis is available in the ICRISAT library.

Materials and Methods

Most observations were made on material from plants grown in the field at the ICRISAT Center; a few samples were also taken from plants grown in pots.

Fixation. The material was fixed for at least 48 hr either in FPA (formalin 40%: propionic acid : ethyl alcohol 70%, in the proportion 5:5:90 by volume) or in glutaraldehyde (3% solution in phosphate buffer, pH 6.8). In this report Figures 20, 21, 22, 29, 32, 53, 54, 55, and 56 are of material fixed in glutaraldehyde; the remaining figures are of material fixed in FPA, unless otherwise stated.

Dehydration and embedding in wax. After fixation the material was dehydrated by immersion in a series of alcohol solutions, followed by a mixture of absolute alcohol and tertiary butanol, and finally brought to pure tertiary butanol. The material was kept in a mixture of tertiary butanol and paraffin wax (melting point 60°C) in an oven for 24-72 hr before infiltration in pure paraffin wax. The material was then embedded in wax.

Sectioning. Sections of the wax-embedded material were cut on a Cambridge Rotary Microtome at a thickness of 8-10 y for soft material such as buds, and 12 u for harder

material. Sections of woody stems and roots were cut on a sliding microtome at a thickness of 12-15 u after the material had been boiled in water to expel air bubbles and to soften it; this material was cut directly without embedding in wax. The sections shown in Figures 3, 4, 5, 6, 7, and 9 were prepared in this way.

Slide preparation. The ribbons of serial sections from the rotary microtome were floated on warm water (50°C) before being arranged on slides, which had been smeared with Haupt's adhesive (1 g Knox gelatine, 2 g phenol, and 15 ml glycerol in 100 ml water). The slides were kept on a hot plate at 50°C and a few drops of 4% formalin were added to coagulate the gelatine in the adhesive.

The slides were dewaxed in pure xylene for 5-60 min before staining. They were passed through 100%, 90%, 70%, and 50% alcohol solutions and finally water before staining in aqueous solutions; for alcoholic stains, this dehydration was not necessary. After staining the slides were dehydrated in alcohol, passed to an alcohol-xylene mixture, and cleared in xylene before mounting in DPX mountant, Permount or Canada Balsam. Some of the sections stained with aniline blue and mounted in DPX faded after several months.

Stains, (a) Toluidine Blue. This relatively new stain is polychromatic, giving different colors with different tissues. Staining was carried out in a 0.05% aqueous solution as described by Feder and O'Brien (1968).

(b) Safranin and Aniline Blue. The slides were first stained in safranin (1% in 70% alcohol), washed in 90% alcohol, and counter-stained in aniline blue (1% in absolute alcohol).

(c) Trypan Blue. For the detection of mycorrhizae in cleared root material we followed the procedure of Phillips and Hayman (1970). After clearing in 10% potassium hydroxide the roots were stained with trypan blue in lactophenol.

(d) Haematoxylene. The sections were kept in a 3% aqueous solution of iron alum for 0.5-1 hr, washed, kept in a 1% aqueous solution of haematoxylene for 0.5-1 hr, washed, and differentiated in iron alum or in a saturated solution of picric acid in 70% alcohol. Some sections were counterstained in safranin, as described above.

(e) Picric Acid and Iodine. Staining for proteins in seeds was carried out with a saturated solution of picric acid in 70% alcohol; after washing they were stained with iodine in potassium iodine solution to reveal starch. The sections were mounted in glycerine.

Clearing of leaves. The procedure was that of Aloni and Sachs (1973). The leaves were boiled in lactic acid before staining in 0.2% lacmoid in lactic acid, followed by immersion in phosphate buffer (pH 7.5) and mounted in 70% sodium lactate. The material shown in Figures 11 and 12 was prepared by this method.

Stomatal preparations. Standard methods of preparing impressions of the epidermis using nail varnish and silicone rubber failed with pigeonpeas, owing to the hairiness

of the leaves. However it was possible to obtain some stomatal impressions of the upper epidermis only, using Quickfix.

An alternative method was devised, using Scotch tape. The tap was firmly fixedon both sides of the leaf, and then gently peeled off. The epidermis stuck to the tape and in this way good peels could be obtained directly. Even better results were obtained by keeping the peels in 100% alcohol to extract chlorophyll, and then passing them through an alcohol series to water and staining them in a 0.05% aqueous solution of toluidine blue for a few minutes. At this stage the epidermal peel could be separated from the Scotch tape and mounted on a slide in water or glycerine. The stomata were stained pink and were clearly visible (Fig. 17). This method can be used for studying the distribution and frequency of stomata, but not for the study of stomatal apertures, since these probably change during the processing of the epidermis.

Anatomical Observations

Steins

The internodes of the stem develop by elongation of the tissue between the leaf initials in the apical meristem (Fig. 1).

The primary vascular tissue of the stem is organized in strands connecting the nodes; each strand is associated with a ridge on the stem. These ridges are clearly visible even in old, secondarily thickened stems. CoUenchymatous bundle caps underlie the epidermis of the ridges (Fig. 2).

Although the xylem and phloem are organized into fairly distinct strands in young stems, the fibers towards the exterior of the phloem are not confined to these primary vascular strands but form a continuous ring. Immediately outside this ring of fibers is a one-cell-thick ring of thin-walled cells, some of which contain rhomboidalcrystals.

The cortex in between the bundle caps is usually three or four cells thick. The vacuoles of some of these cells stain densely with most stains, and in unstained fresh material turn blue soon after sectioning. At a later stage some of the cortical cells contain reddish anthocyanin pigment in their vacuoles. It is possible that the densely staining material in the vacuoles of young cortical cells is an anthocyanin precursor.

The epidermis of the stem bears hairs of the same types that are found on leaves, described below.

The stem thickens as a result of the activity of the vascular cambium, which produces a continuous ring of xylem tissue towards the inside and phloem towards the outside (Fig. 2). The continuation of these processes results in thick woody stems (Figs. 3 and 4). In the outer part of the bark there are well-developed lenticels (Fig. 5).

Xylem. Within the xylem the vessels are either solitary or in radial or, more rarely, tangential multiples (Fig. 3). The vessels are surrounded by parenchymatous cells, and tangential bands of parenchyma run between the vessels (i.e., in an aliform-confluent pattern). Much of the remainder of the xylem tissue between the medullary rays consists of xylem fibers.

The medullary rays are either one cell thick (uniseriate) or several cells thick (multiseriate). Medullary rays can be seen in transverse section in Figures 3, 4, and-8, in tangential longitudinal section in Figure 6, and in radial longitudinal section in Figure 7.

During the vegetative phase the parenchymatous cells in the xylem, including the medullary rays, contain large quantities of starch (Fig. 8). These starch reserves can be detected very simply in the field, by applying a few drops of iodine to the cut ends of stems or branches, which turn blue or black if starch is present. During the reproductive phase, these starch reserves disappear; but in plants from which flowers are continuously removed, the starch reserves in the stem are not depleted, indicating that these reserves are mobilized as a consequence of pod development.

Tension wood. During the monsoon season the strong westerly winds bend the stems and affect the pattern of branching, with a permanent effect on the morphology of the plants (see Narayanan and Sheldrake 1975, chapter 8). The bending of the stems results in an asymmetric development of wood, which becomes thicker on the windward (west) side of the stem. Within the xylem on this side, bands of fibers with gelatinous thickenings are formed (Fig. 9). Such thickenings are characteristic of "tension wood" in dicotyledons.

Secretory ducts. Within the phloem region of the stems and also in the outer part of the pith near to the primary xylem tissue (Fig. 2) are cells containing material that stains densely with all the dyes we have used; even in unstained sections the contents of these cells appear reddish brown. In longitudinal sections these cells are seen to be elongated and joined end to end to form ducts (Fig. 5). These cells differentiate at an early stage within primary tissues (Figs. 1 and 10) and are also formed within secondary phloem tissue (Fig. 4). They are found in leaves, petioles, roots, flowers, and pod walls; indeed they occur throughout the plant body.

When young tender shoots are cut, a drop of fluid exudes onto the cut surface. This fluid, which has an extremely astringent taste, is colorless at first, but turns red on exposure to the air. The result of this process is that wounds on stems, leaves pods, etc., become covered with red material that dries to form a sort of varnish or, if exuded in greater quantities, transparent globules. This solid material, like the fluid from which it is derived, has a bitter astringent taste.

The chemical nature of this material is unknown, but it seems probable that it contains tannin-like polyphenolic compounds, which are oxidized on exposure to the air. Possibly it plays a role in protecting the plant against pests and/or diseases.

Leaves

The leaves are trifoliate. The pattern of the main veins within the lamina is clearly visible to the naked eye. Within the areas enclosed by the minor veins are veinlets containing a single file of tracheids, many of which end blindly (Fig. 11).

In the midrib of the leaf, the vascular tissue in the ventral half occurs in a continuous arched band, with phloem on the outside and xylem within. In the ventral part, there are two distinct strands mostly consisting of phloem tissue. The center of the dorsal part of the midrib is occupied by fibers, above which is a cap of coUenchymatous cells (Fig. 13). Crystals are frequently seen in the cortical cells immediately adjacent to the fibers. These are clearly visible in minor veins, especially in polarized light (Fig. 12). Smaller crystals are found within the phloem parenchyma.

In the leaf lamina there is a distinct palisade layer, and in the lower part of the leaf a spongy mesophyll with large air-filled intercellular spaces (Fig. 14).

Hairs. The leaves of pigeonpeas are pubescent, more so on the lower than on the upper surface. At low magnification, two distinct types of hair can be seen: simple, and glandular (Fig. 15). The glandular hairs are spherical and yellow in color. In fresh sections of leaves, they resemble small balloons, distended with yellow fluid (Fig. 16). When these hairs are damaged, the contents escape and a deflated, colorless sac remains. These sacs appear to contain an oily secretory material, which is probably responsible for the fragrance of the vegetative parts of the pigeonpeas. Essential oil can be collected by the steam distillation of leaves, etc., of pigeonpeas; it contains a mixture of compounds including the terpenoid a-Copaene (Gupta et al. 1969).

These fluid-filled sacs seem to develop from short multicellular glandular hairs found on young leaves (Fig. 17).

The mean frequency of these yellow-fluid-filled glandular hairs on young mature leaves of 10 medium- and late-duration cultivars was 1.9/mm^2 on the upper and 3.2/mm^2 on the lower surface. The number per leaf declined with age, presumably owing to the damage and rupture of the hairs. The variation in number from leaf to leaf within a cultivar was too great to enable cultivaral differences in hair frequency to be established.

Similar simple and glandular hairs are found on all aerial parts of the plants, except some parts of the flowers such as petals and stamens. In addition, a further type of glandular hair is very frequent on pod walls (Fig. 43) but is rarely seen on other organs.

Stomata. There are over 10 times as many stomata on the lower than on the upper surface of the leaves (Table 1).

Table 1. Frequency of stomata on the upper and lower epidermis of fully expanded leaves.

Cultivar	Number of stomata/mm^2	
	Upper surface	Lower surface
ICP-1	55 +33.2	667 + 34.4
T-21	43 +44.4	609 + 103.9
HY-3C	19 +17.1	447 + 94.3

Stomata in the lower epidermis of a leaf are shown in Figure 18. We have not so far been able to make impressions of this epidermis in silicone rubber or other material, owing, to hairiness of the surface. However, satisfactory impressions of the upper epidermis were obtained with Quickfix (Fig. 19).

Petioles. The petiole contains a number of distinct vascular strands (Fig. 20) outside which lie bands of fibers (Fig. 21). The two "flanges" projecting from the upper surface of the petiole contain small vascular bundles. Not uncommonly a few of the xylem vessels in petioles were found to be filled with dark staining material resembling the tanninlike material in secretory ducts (Fig. 21). We do not know the reasons for this.

Pulvini. At the proximal end of the petiole is a swollen region, the pulvinus. Similar, but smaller, pulvini are found at the junction between each leaflet and the petiole.

The changes in turgor on different sides of these pulvini are responsible for the movements of the leaves and petioles. The canopy structure of pigeonpeas is constantly changing as a result of these pulvinar movements. The upright steep positions of the leaves at night depend on the geotropic sensitivity of the pulvini, and the movements of the leaves throughout the day are influenced by intensity of the light falling on the pulvini and also the water status of the plant (see Narayanan and Sheldrake 1975, chapter 8).

The vascular tissue is arranged in a horseshoe shape in the center of the pulvinus, with the xylem on the inside; the phloem is surrounded by a band of fibers (Fig. 22). As in the stems, leaf veins, and petioles, small crystals (presumably of calcium oxalate) are found scattered throughout the phloem, in parenchymatous cells; larger crystals are found in the cortical cells adjacent to the phloem fibers.

Most of the pulvinus consists of cortical tissue. Many of the cells in the cortex contain densely staining tanninlike material within their vacuoles (Fig. 22). It is the changes in the turgor of these cortical cells that are responsible for the movements of the pulvini. In other species, such changes have been shown to be due to active fluxes of $K+$ and other ions.

Abscission zones. In senescent leaves an abscission zone develops at the junction between each leaflet and the petiole and at the junction between the petiole and the stem. In each case the abscission zone is proximal to the pulvinus.

Prior to abscission, cells in the abscission zone divide, with the plane of cell division parallel to the plane of abscission. The weakening of the walls of these cells (which in other species has been shown to depend on the production of cellulase and other hydrolytic enzymes) results in an easy separation of the two sides of the abscission zone (Fig. 23). Consequently the leaflet or petiole falls.

Abscission zones are also formed at the juction between the peduncle of the inflorescence and the pedicel of flowers or young pods (Fig. 24). In pigeonpeas, pods develop from only a small minority of the flowers (see Sheldrake et al. 1979); most of the flowers abscind infructuously.

Roots

The primary structure of the roots is usually tetrach (Fig. 25). Secondary thickening takes place as a result of the activity of the cambium. In older roots, there are numerous rhomboidal crystals in cells of the cortex (Fig. 26). As in stems and other aerial organs, secretory ducts containing tanninlike material are present within the phloem region.

Mycorrhizae are often present in cortical cells of the roots (Fig. 27) and occasionally fruiting bodies of the mycorrhizae can be observed (Fig. 28).

Nodules. In the young nodules, growth occurs from a meristematic zone arching around the apical end (Fig. 29). The medulla of the nodules contains numerous bacterioid-filled cells (Figs. 30 and 31). Vascular stands are present in the cortex (Figs. 29 and 30). Sometimes the bacterioidal cells are highly vacuolated, as in Figure 29, but the nonvacuolate form shown in Figures 30 and 31 is more usual. We do not know the cause of this vacuolation.

In some nodules bacterial infection threads are clearly visible in cells near the meristematic zone. These infection threads appear to move through the cells (Fig. 32).

Reproductive Structures

The development of flowers. An early stage in the development of flower buds is shown in longitudinal section in Figure 33 and a late stage in Figure 34. Earlier and later stages are shown in tranverse section in Figures 35 and 36, respectively.

Pollen mother cells develop and separate from each other within the embryonic anther (Fig. 37). Their nuclei undergo meiosis, resulting in the formation of tetrads containing four nuclei (Fig. 38); then tetrads separate into four distinct cells (Fig. 39), which develop into pollen grains (Fig. 40).

We have not studied the process of megasporogenesis in detail. We think that the stage shown in Figure 41 represents the cells derived from the megaspore mother cell by meiosis. One or more of these cells gives rise to the embryo sac (Fig. 42), and the others degenerate.

The development of pods. In the first 3 weeks after anthesis, the pod wall grows more rapidly than the young seeds, but thereafter undergoes little further growth (see Narayanan and Sheldrake 1975, chapter 4).

The pod wall is well supplied with secretory ducts containing tanninlike material (Fig. 43). The outer epidermis of the pod contains stomata. This epidermis bears simple hairs and globular secretory hairs containing yellow oil (Fig. 44) similar to those found on leaves (Figs. 15-17). In addition, there are large numbers of a third type of hair seen only occasionally on vegetative organs, with secretory cells towards the base and a long tubular neck (Fig. 43). These hairs produce a colorless liquid exudate, which in fresh material can be seen in droplets at the tips and on the outside of the hairs.

At the time of anthesis, the ovules are present in an undivided space within the carpel (Fig. 34), but within the 1st week of pod development cross-walls develop between the developing seeds, dividing the pod into locules (Fig. 46).

During the 1st week after anthesis the endosperm undergoes rapid development and becomes the predominant tissue in the developing seed (Figs. 45 and 46); by the end of the 2nd week there are still large amounts of endospermous tissue (Fig. 48). Within the embryo, distinct cotyledons are apparent (Fig. 47). Further development of the seeds involves rapid growth of the cotyledons and degeneration of the remaining nutritive tissues.

Pedicels. The pedicels of the flowers contain small vascular bundles surrounded by a ring of fibers (Figs. 49 and 50). During the 1st week of pod development, secondary thickening begins and the cambium gives rise to a ring of xylem tissue towards the inside of the pedicel and to a ring of phloem towards the outside (Fig. 51). Secondary thickening continues during the 2nd and 3rd weeks but slows down in the 4th week, after which little more vascular tissue develops. This thickening results in a great increase in the cross-sectional area of the vascular tissue supplying the pod (Fig. 52). During the later stages of pod development the phloem tissue collapses, probably as a result of the maturation and desiccation of the pod.

The phloem fibers, like the xylem fibers, contain large amounts of gelatinous wall thickening and do not become heavily lignified.

Some of the cells of the pith, at first typically thin-walled, begin to show reticulate wall thickenings by the end of the 2nd week of pod development. Wall thickening takes place in more pith cells during the 3rd week, and some of these cells begin to lignify. By the time of maturation most of the pith cells possess thickened, pitted, lignified walls resembling those of xylem tracheids.

Seeds

The large cotyledons of the embryo fill most of the seed (Fig. 53). The seed coat resembles that of other legumes, with an outer palisade layer of sclereids and a subepidermal layer of "pillar cells," between which are large intercellular spaces (Fig. 54). A thin layer of collapsed parenchymatous cells and the remains of the endosperm underlie the subepidermal cells.

At the hilum region, a hole in the seed coat leads into a "tracheid island" (Fig. 55). The tissue outside the seed coat at the hilum is known as funicular tissue; this tissue includes a palisade layer adjacent to that of the seed coat (Fig. 55).

The parenchymatous cells of the cotyledons contain large starch grains and numerous protein bodies (Fig. 56). Vascular strands run throughout the ground tissue of the cotyledons.

After germination of the seeds, the protein bodies and starch grain within the cotyledons gradually disappear as the reserves are mobilized into the growing seedlings; the cotyledons are exhausted within a week or so.

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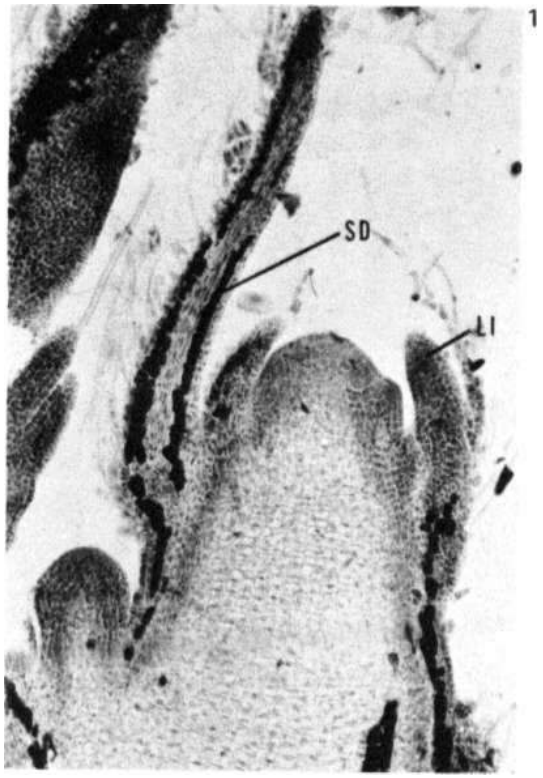
Figures

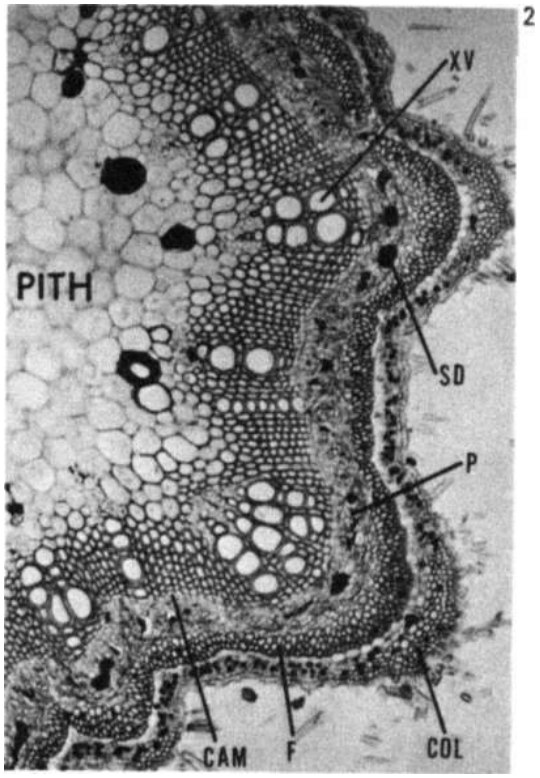
Abbreviations used in the figures

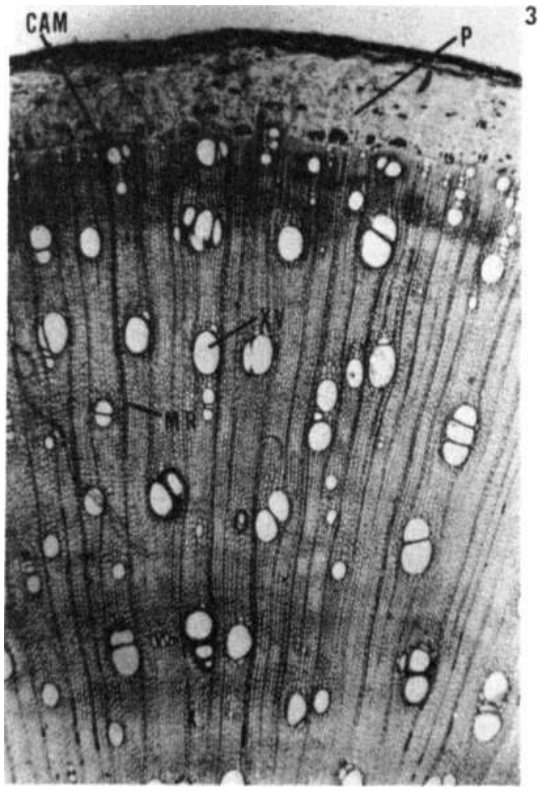
A	Abscission zone
AN	Anther
B	Bacterioid
CAM	Cambium
COL	Collenchyma
COT	Cotyledon
CP	Carpel primordium
CRY	Crystal
EMB	Embryo
END	Endosperm
EP	Epidermis
F	Fiber
FB	Fruiting body
FT	Funicular tissue
GH	Glandular hair
H	Simple hair
LI	Leaf initial
M	Mycorrhizae
MR	Medullary ray
MZ	Meristematic zone
O	Ovary
P	Phloem
PAL	Palisade tissue
PAR	Parenchyma
PC	Pillar cell
PET	Petal
PG	Pollen grain
PMC	Pollen mother cell
S	Stomata
SCL	Sclereids
SD	Secretory ducts containing tanninlike material
SM	Spongy mesophyll
SP	Stamen primordium
ST	Starch
T	Tetrad
TAP	Tapetum
VB	Vascular bundle
X	Xylem
XF	Xylem fiber
XV	Xylem vessel

Abbreviations used in the figure caption

A.B.	Aniline blue
Haem.	Haematoxylene
L.S.	Longitudinal section
Saf.	Safranin
T.S.	Tranverse section
Tol. B.	Toluidine blue







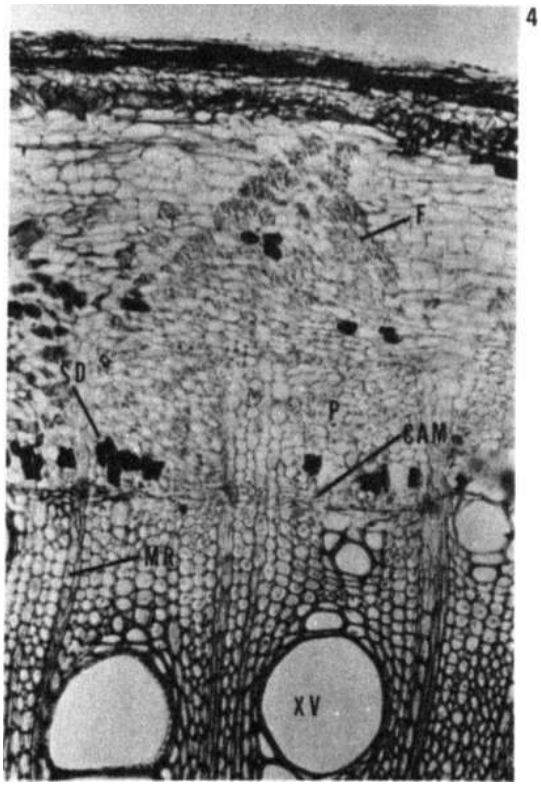


Fig. 1 : L.S. apical meristem, cv. ICP-1 (Saf. - A.B.) x 113.

Fig. 2 : T.S. young secondarily thickening stem, cv. ST-1 (Saf. - A.B.) x 75.

Fig. 3 H T.S. woody main-stem, cv. Hy-3C (Saf. - A.B.) x 30.

Fig. 4 : T.S. woody main-stem, bark region, cv. HY-3C (Saf. - A.B.) x 113.

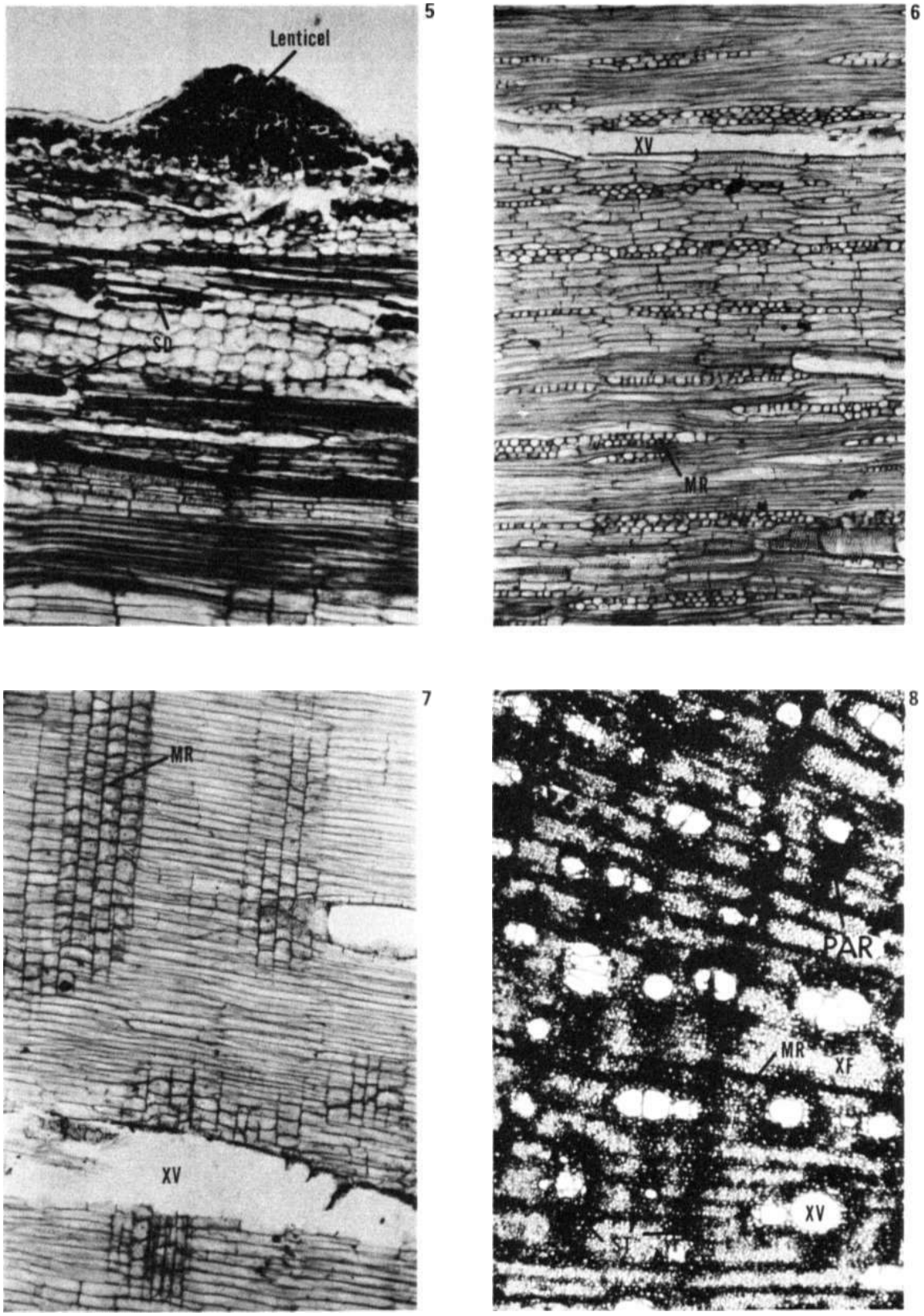
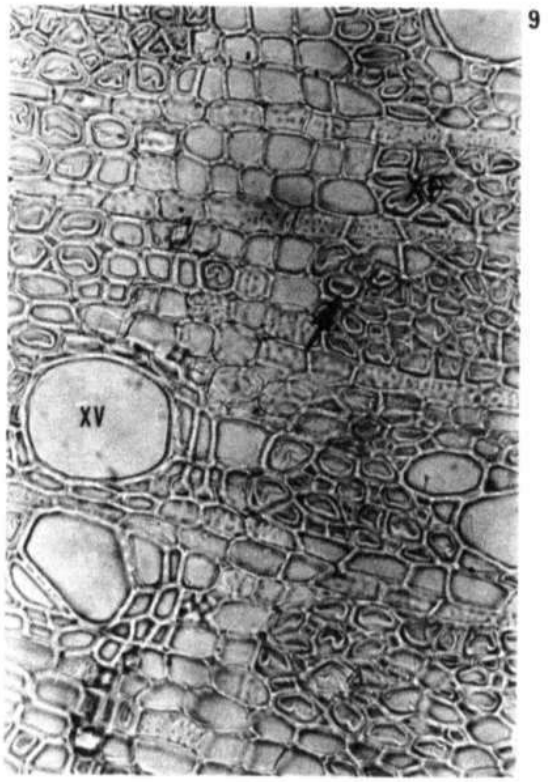


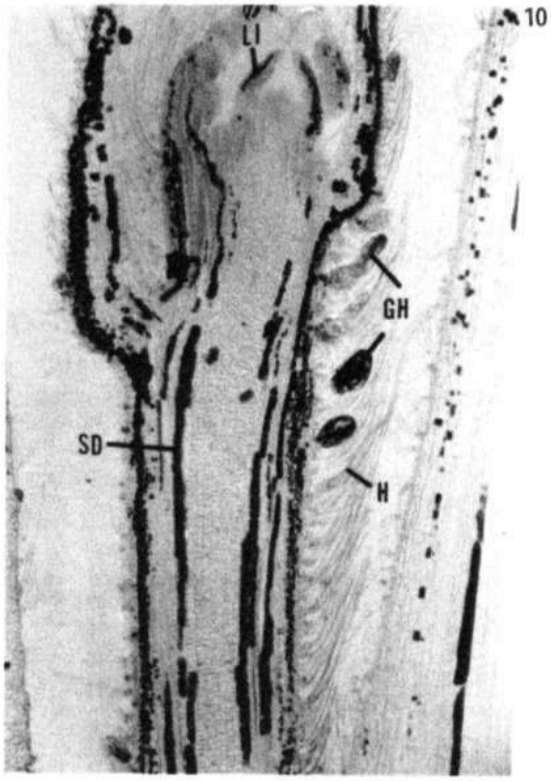
Fig. 5: L.S. bark region of woody stem, showing lenticel, cv. ICP-1 (Tol. B) x 113.

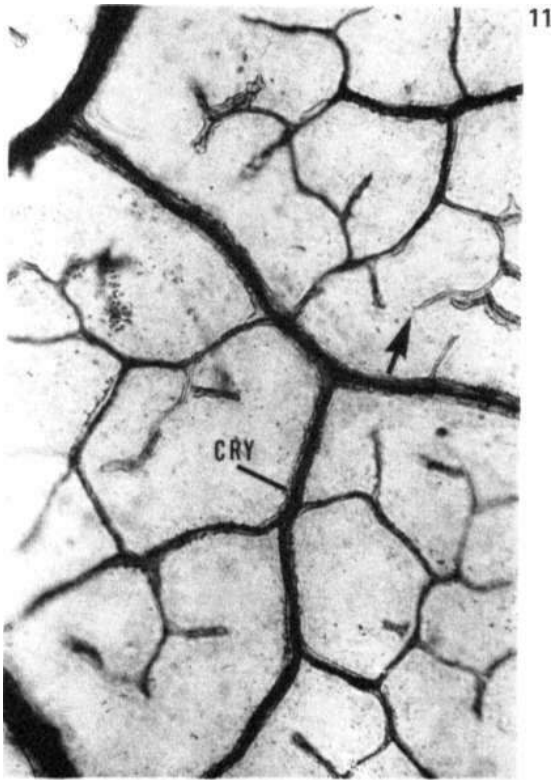
Fig. 6: Tangential L.S. of xylem in woodv stem, showing medullary rays, cv. ST-1 (Tol. B) x 75.

Fig. 7: Radial L.S. of xylem in woody stem showing medullary rays, cv. ST-1 (Tol. B) x 75.

Fig. 8: T.S. xylem in woody stem stained with iodine, showing starch in medullary rays and xylem parenchyma, cv. ST-1 x 75.







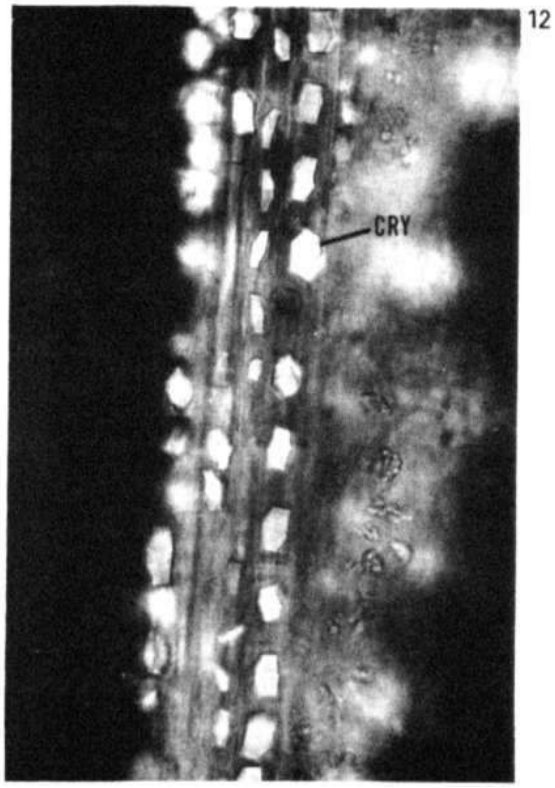
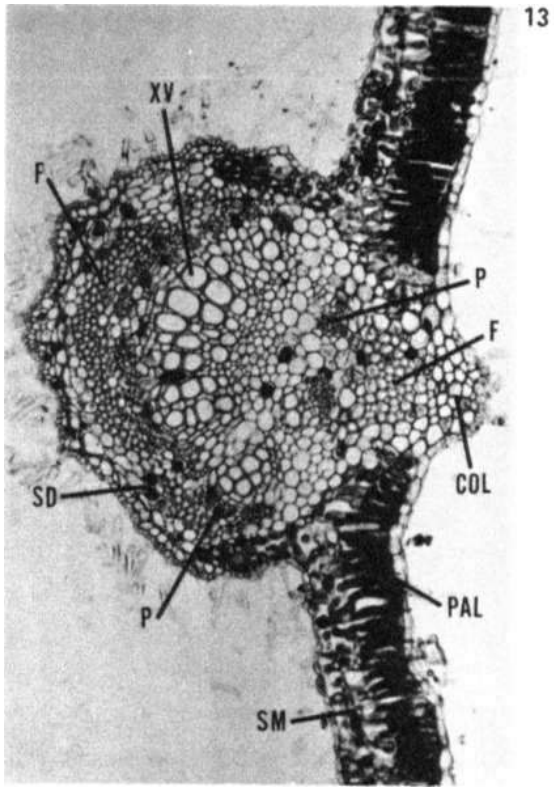


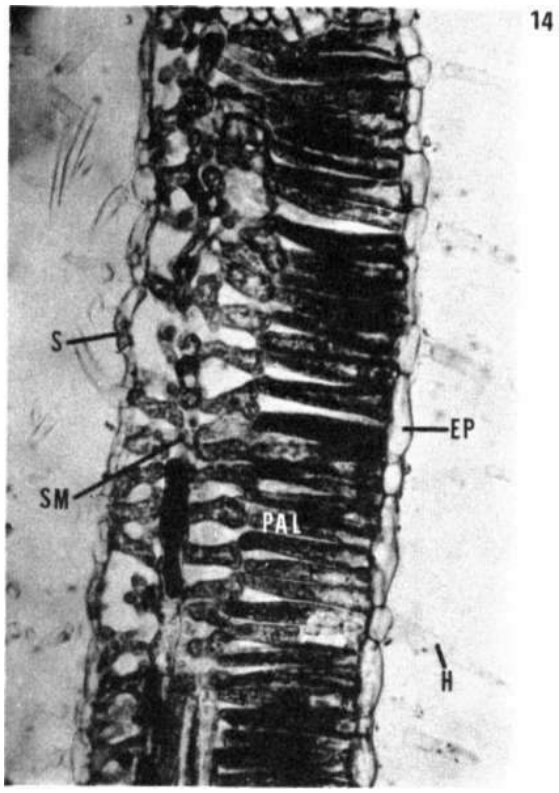
Fig. 9 : T.S. xylem in woody stem showing gelatinous thickening (arrow) in xylem fibers, cv. ST-1 (Saf. - A.B.) x 300.

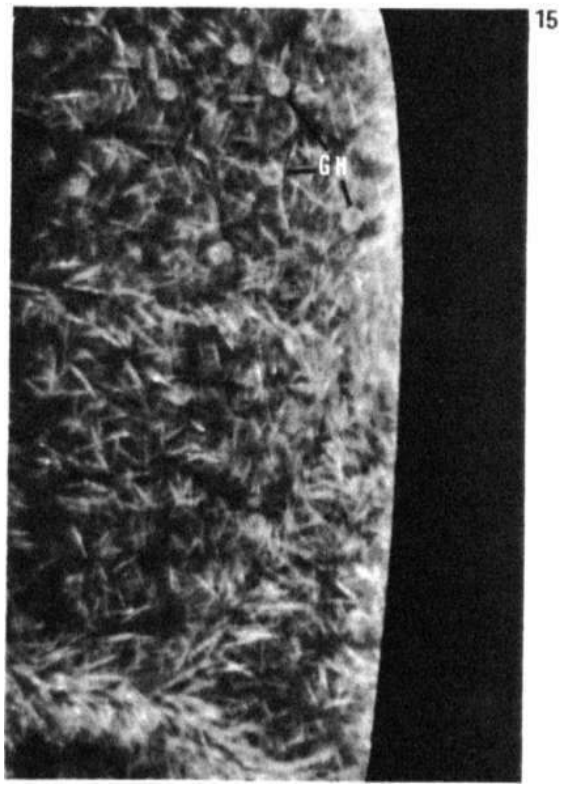
Fig. 10 : L.S. axillary bud, showing secretory ducts, cv. T-21 (unstained) x 75.

Fig. 11 : Cleared leaf showing veins; many minor veins end blindly (arrow), cv. HY-3C (stained with lacmoid in lactic acid) x 300.

F1g. 12 : Cleared leaf photographed in polarized light showing crystals associated with the veins. cv. HY-3C x 375.







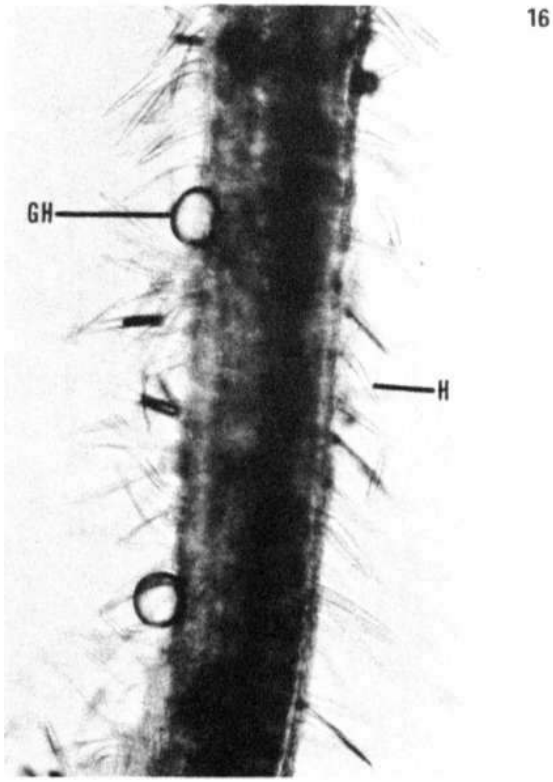
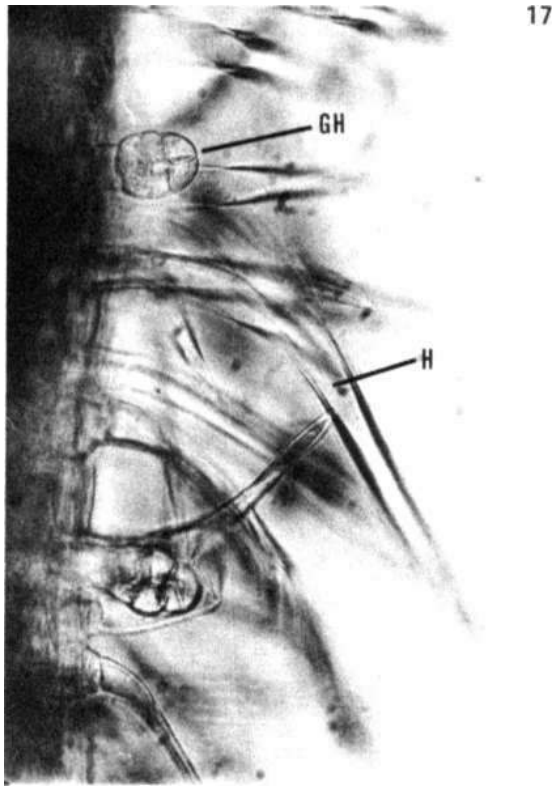


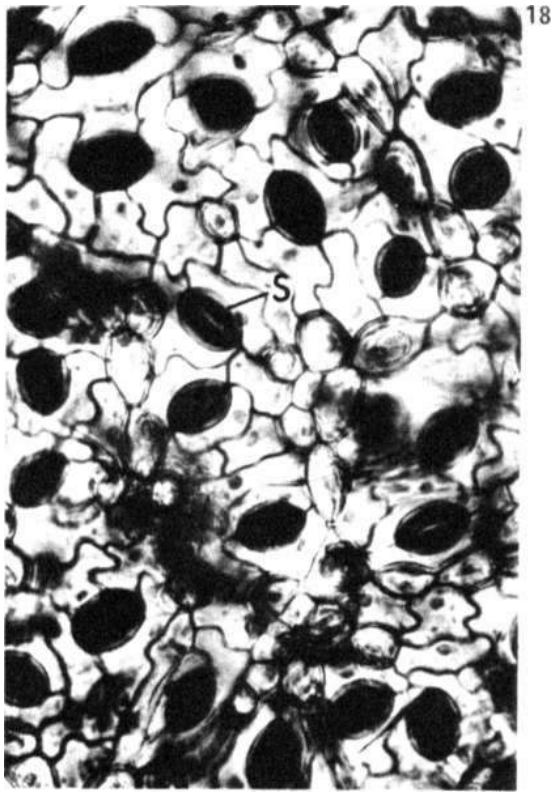
Fig. 13 : T.S. leaf in mid-vein region, cv. ST-1 (Saf. - A.B.) x 113.

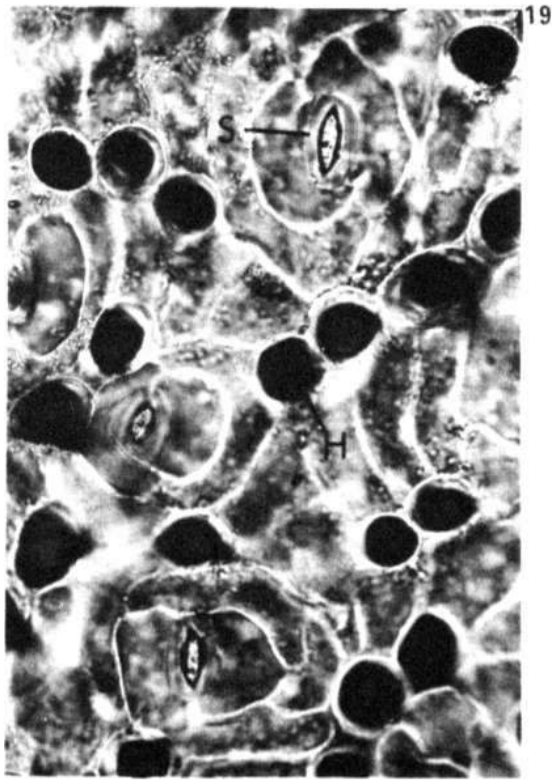
Fig. 14 : T.S. leaf lamina, showing palisade tissue and spongy mesophyll, cv. ST-1 (Saf. - A.B.) x 375.

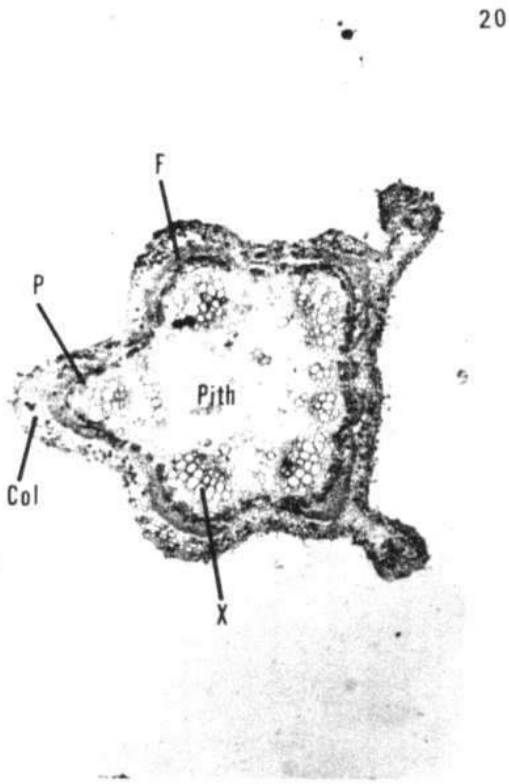
Fig. 15 : Surface view of edge of the lamina of a fresh leaf showing spherical oil-filled glandular hairs, cv. HY-3C x 45.

Fig. 16 : T.S. fresh leaf lamina showing simple hairs and spherical oil-filled glandular hairs, CV.HY-3C (unstained) x 75.









F1g. 17 : T.S. fresh leaf lamina showing glandular hairs, cv. HY-3C (unstained) x 450.

Fig. 18 : Peel of lower epidermis of leaf lamina showing stomata, cv. ICP-6997 (Tol. B.) x 450.

Fig. 19 : Quickfix impression of upper epidermis of leaf lamina, showing stomata, cv. Pusa ageti x 450.

F1g. 20 : T.S. petiole, cv. T-21 (Tol. B.) x 38.

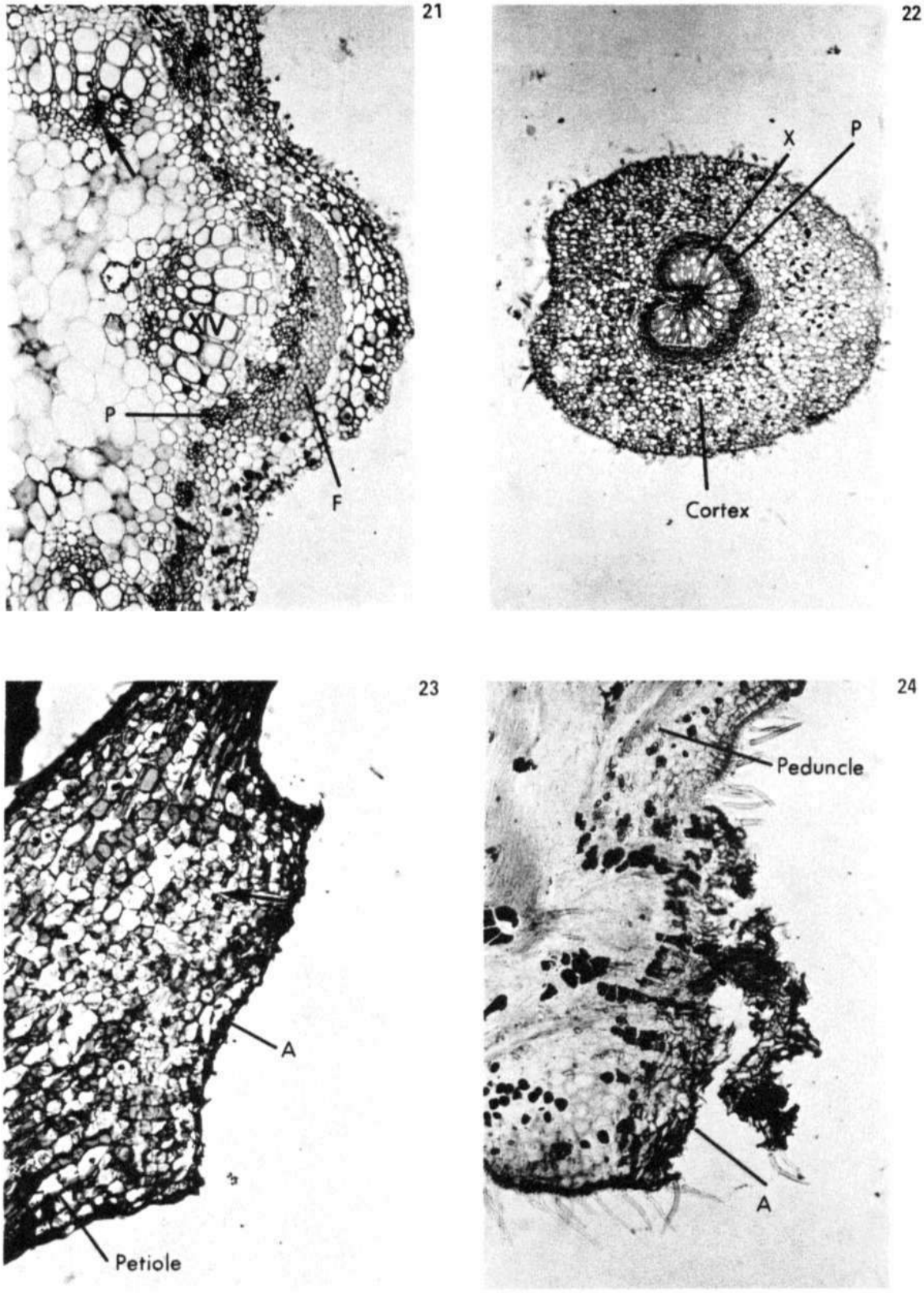
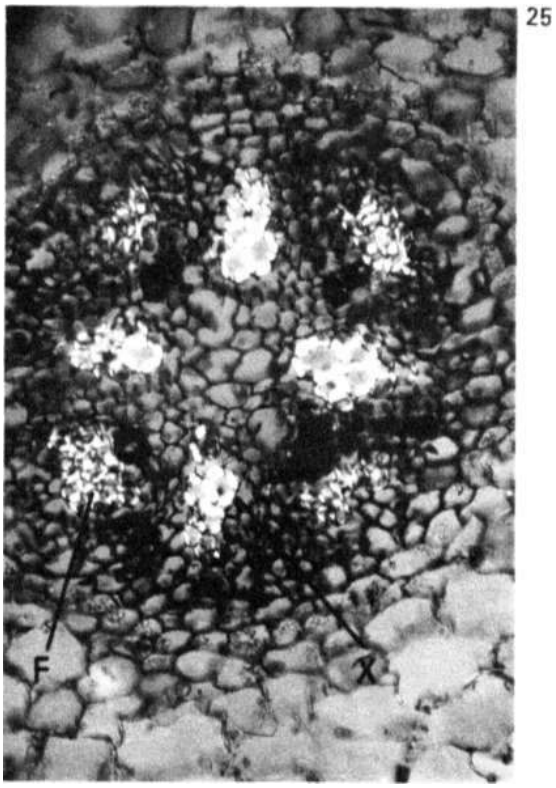


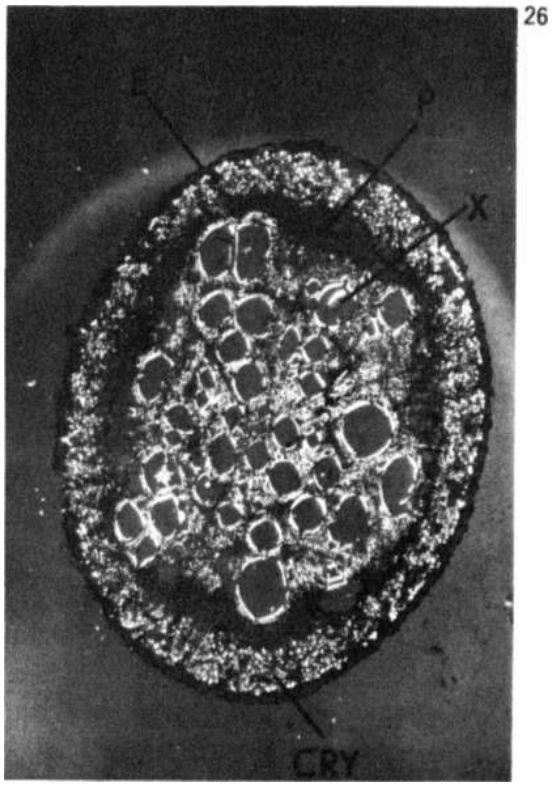
Fig. 21 : T.S. petiole, showing vascular bundles. Some xylem vessels are filled with densely staining material (arrow), cv. T-21 (Tol. B) x 113.

Fig. 22 : T.S. petiolar pulvinus, cv. T-21 (Tol. B) x 45.

Fig. 23 : L.S. abscission zone on petiole after the abscission of a leaflet. Note cell divisions parallel to surface (arrow), cv. HY-3A (Tol. B.) x 75.

Fig. 24 : L.S. abscission zone at base of pedicel after the abscission of a flower, cv. HY-3C (Tol. B.) x 94.





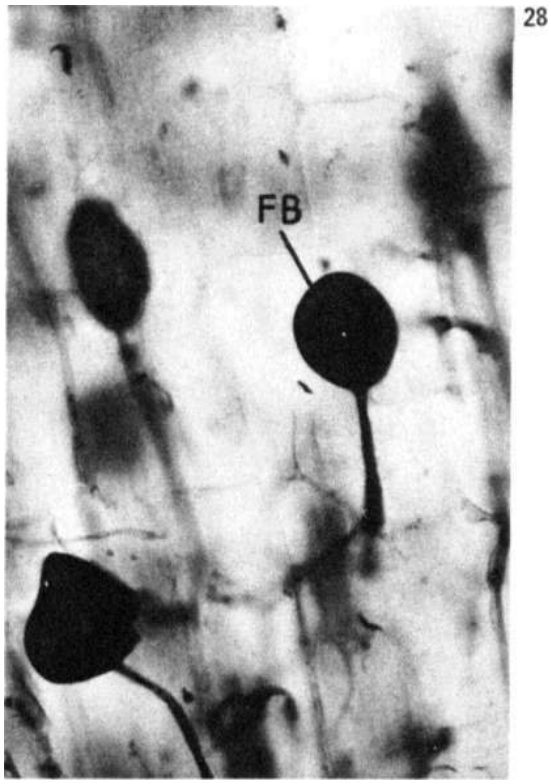
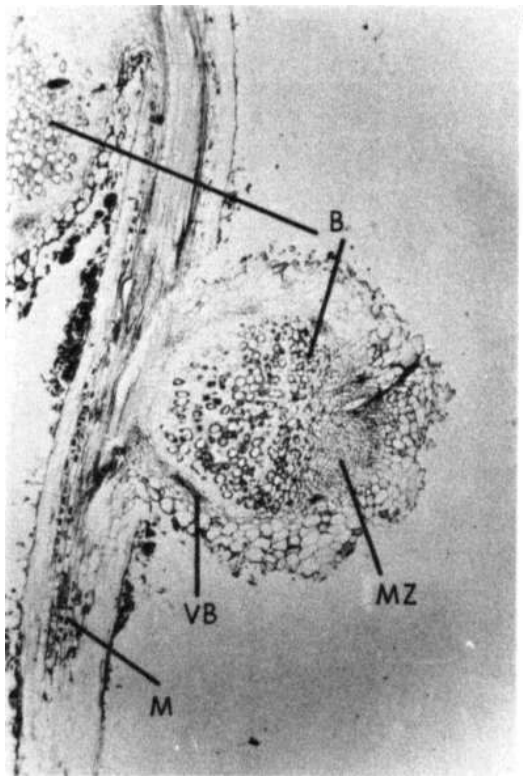


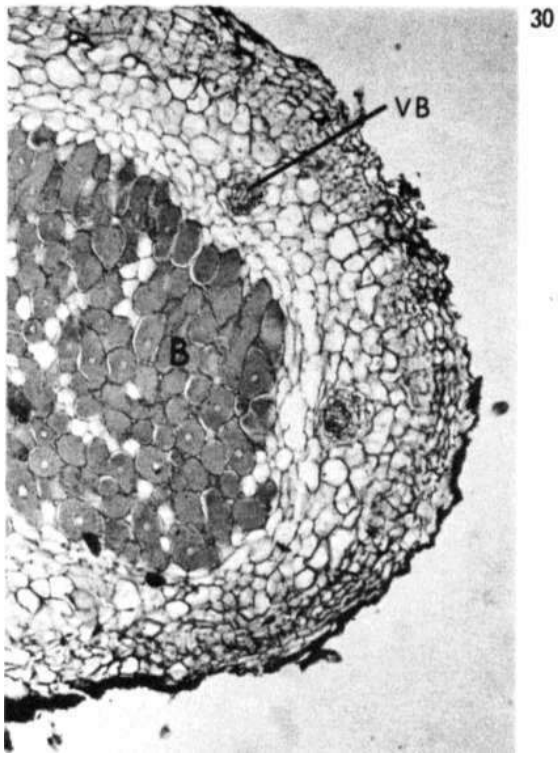
Fig. 25 : T.S. stele of young root showing tetrach arrangement of primary xylem, photographed in polarized light, cv. ICP-1 (Tol.B.) x 300.

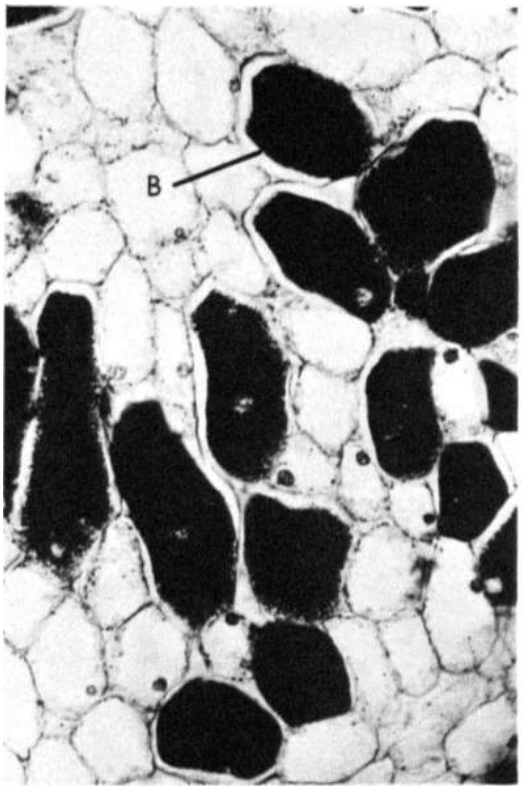
Fig. 26 : T.S. secondarily thickened root, with numerous crystals in the cortex, photographed in polarized light, cv. ST-1 (Tol. B.) x 38.

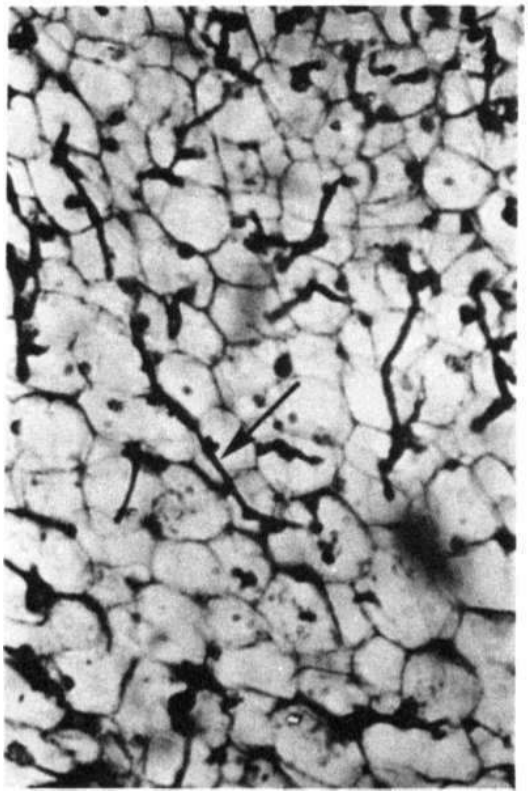
Fig. 27 : L.S. cortex of root, showing mycorrhizae within the cells, cv. ICP-1 (Tol. B.) x 450.

Fig. 28 : Squashed cleared root showing fruiting bodies of mycorrhizae in the cortical region (trypan blue) x 450.







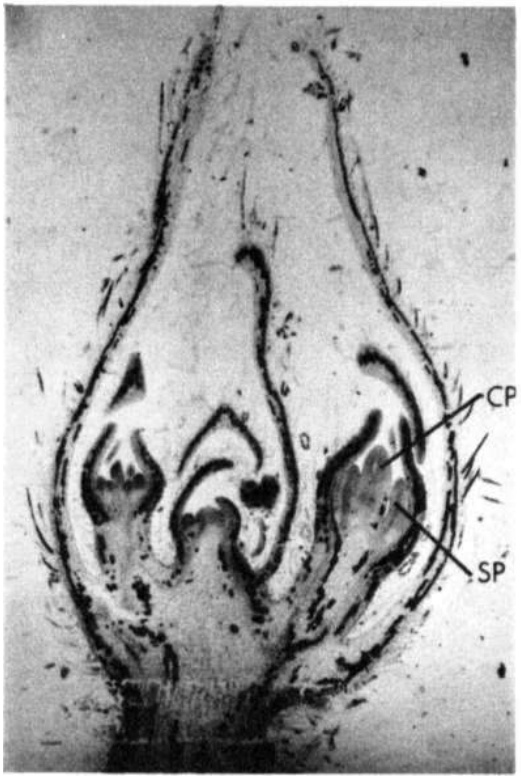


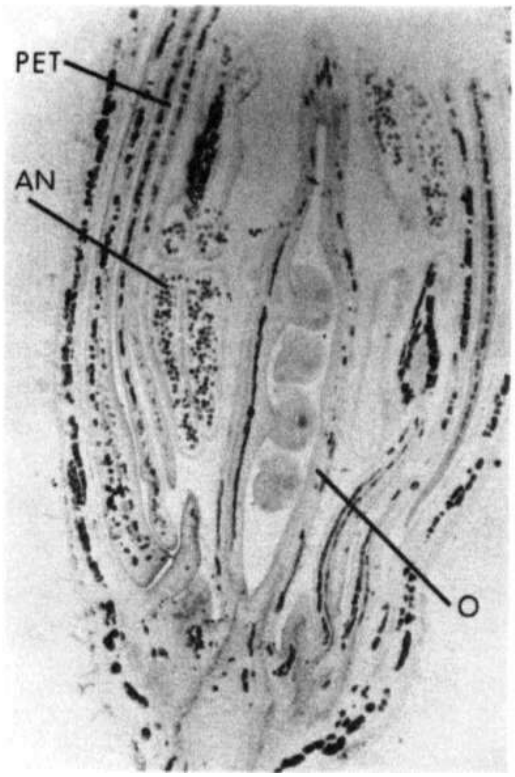
F1g. 29 : L.S. root bearing two nodules. In the nodule on the right the meristematic zone can be seen. The bacterioidal cells are vacuolated. The cortex of the root contains mycorrhizae. cv. ICP-1 (Tol. B.) x 30.

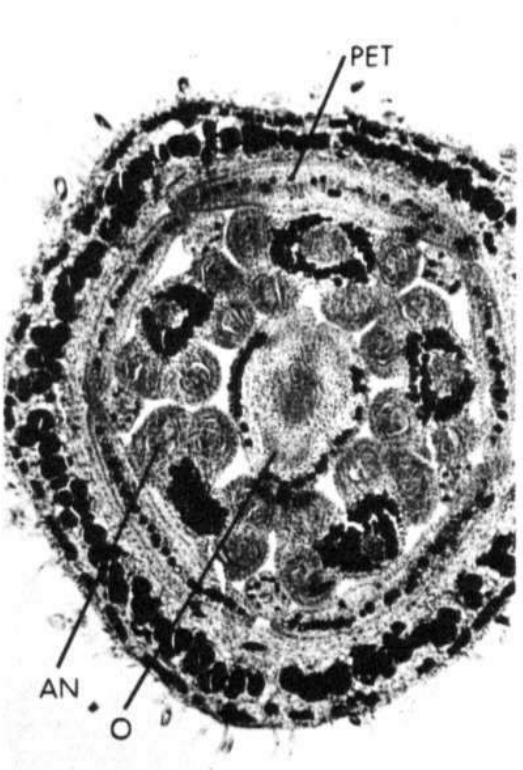
Fig. 30 : L.S. nodule showing bacterioidal cells, cv. HY-3C (Saf. - A.B.) x 75.

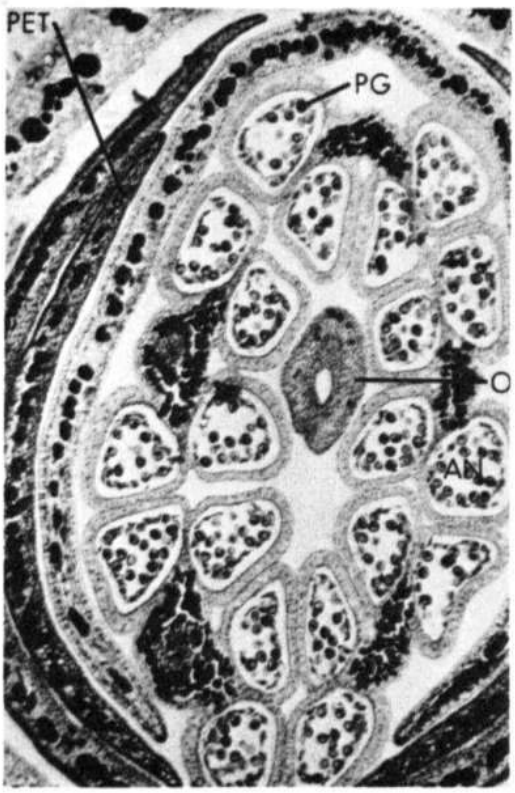
Fig. 31 : L.S. nodule showing bacterioidal cells, cv. HY-3C (Saf. - Haem.) x 300.

Fig. 32 : L.S. cortical region of nodule showing Rhizobium infection threads passing through cells (arrow), cv. ICP-1 (Tol. B.) x 450.







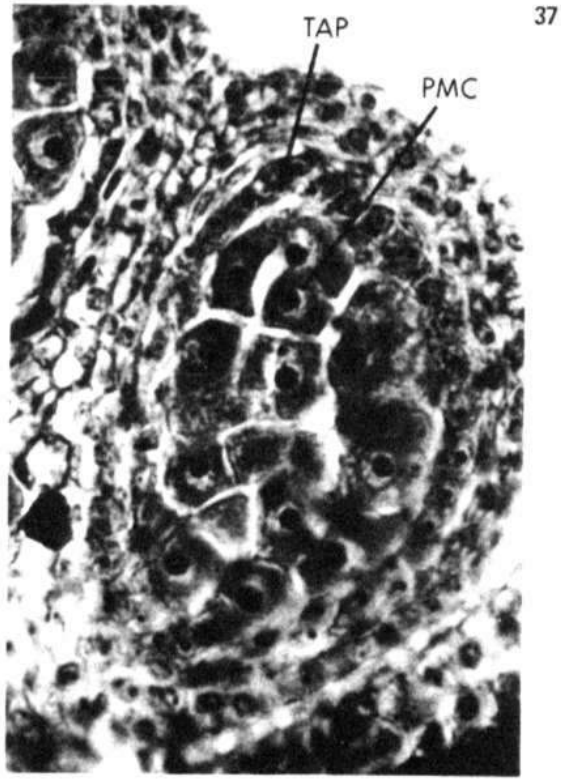


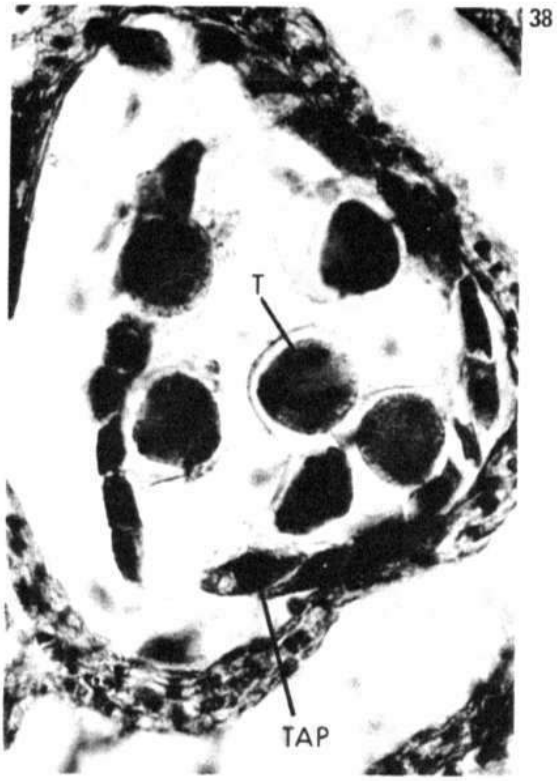
F1g. 33 : L.S. young flower buds showing stamen, carpel and petal primordia, cv. ST-1 (Saf. - A.B.) x 38.

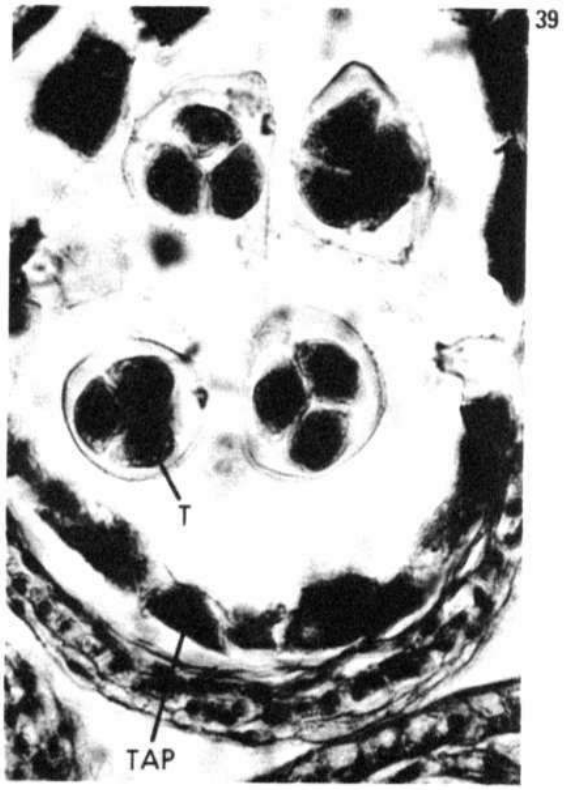
Fig. 34 : L.S. flower bud soon before anthesis, cv. ST-1 (Tol. B.) x 38.

F1g. 35 : T.S. young flower bud showing developing anthers and ovary, cv. ICP-1555 (Haem), x 113.

F1g. 36 : T.S. flower bud soon before anthesis, cv. ICP-1555 (Tol. B.) x 75.







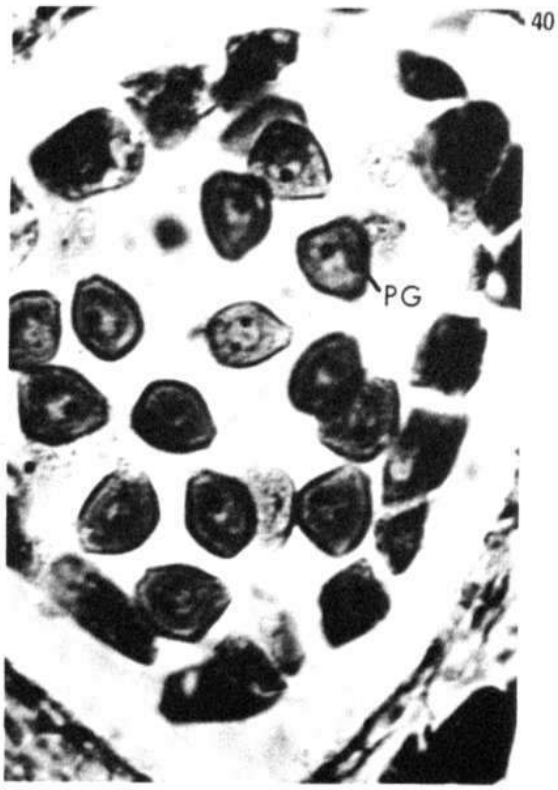
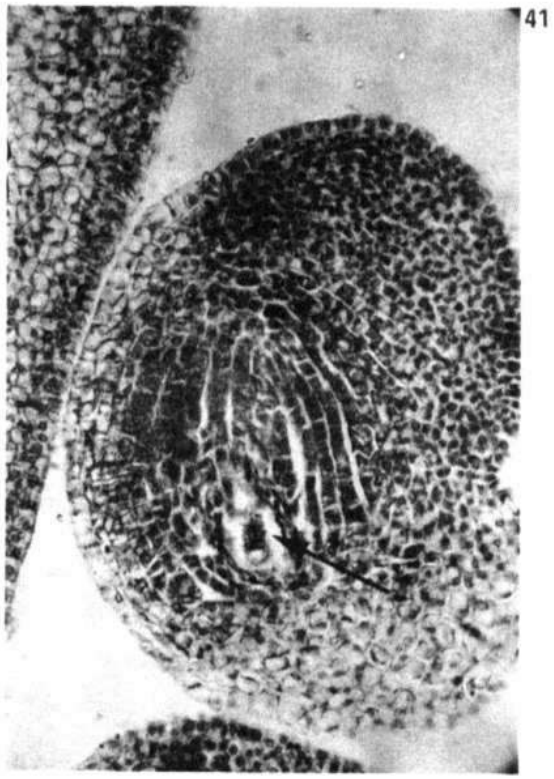


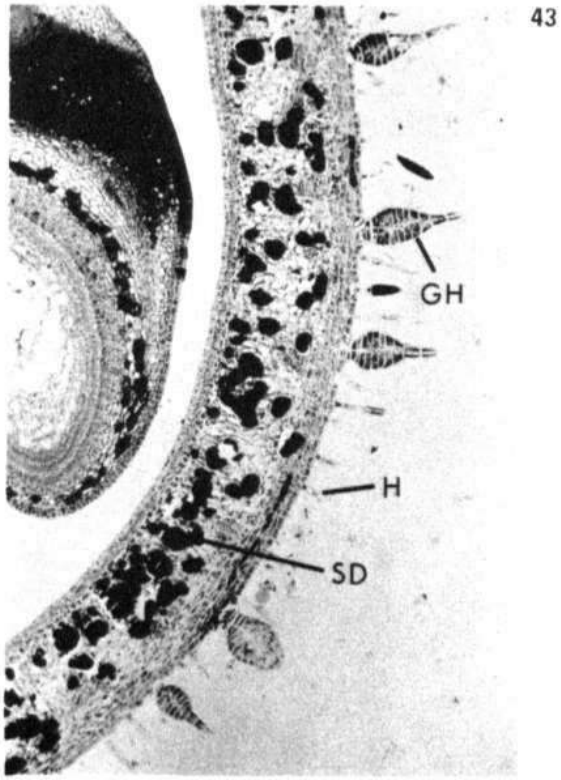
Fig. 37 : T.S. young anther showing pollen mother cells, cv. ICP-1555 (Haem.) x 750.

Fig. 38 : T.S. developing anther showing early tetrads, cv. ICP-1555 (Haem.) x 600.

F1g. 39 : T.S. developing anther showing tetrads, cv. ICP-1555 (Haem.) x 600.

F1g. 40 : T.S. anther soon before anthesis containina pollen qrains cv. ICP-1555 (Haem.) x 300.





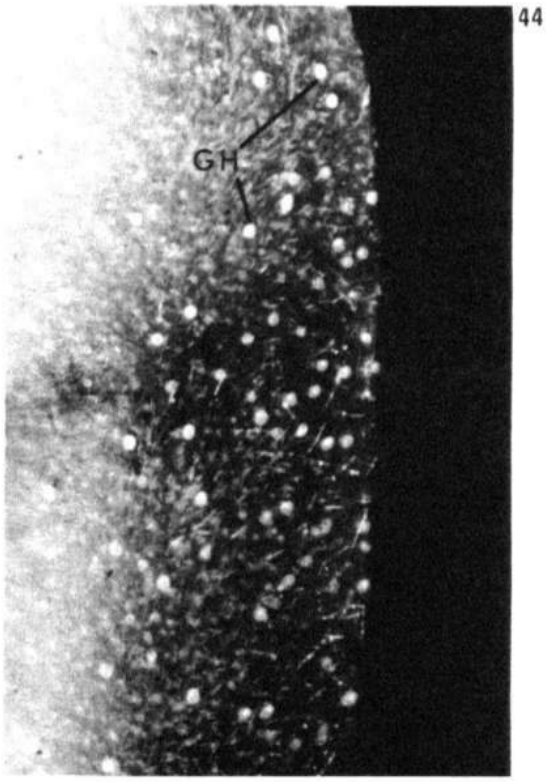
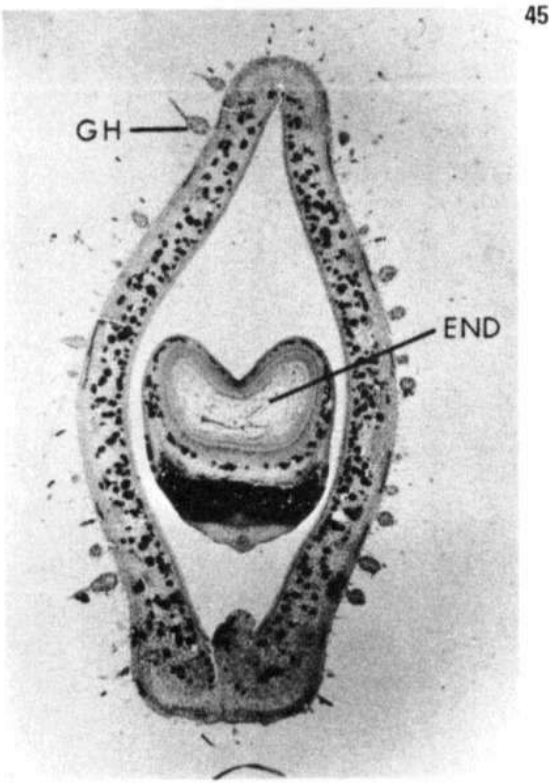


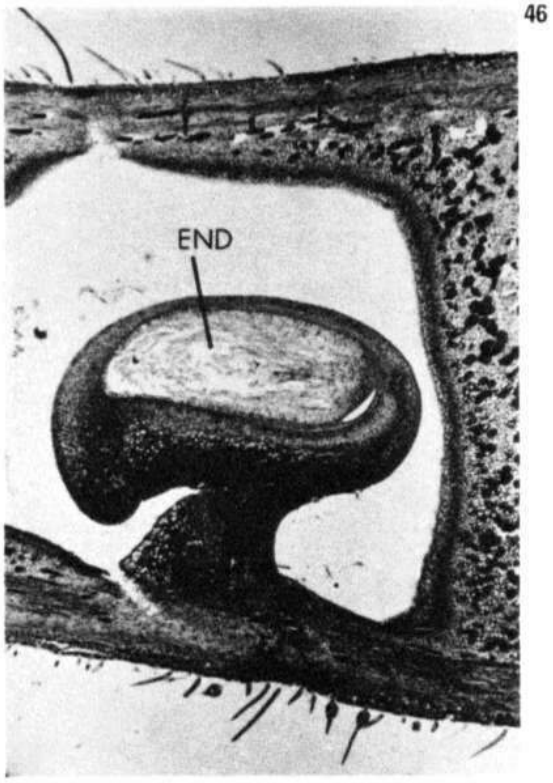
Fig. 41 : L.S. developing ovule showing the cells derived from the megaspore mother cell by meiosis (arrow), cv. ICP-1555 (Tol. B.) x 113.

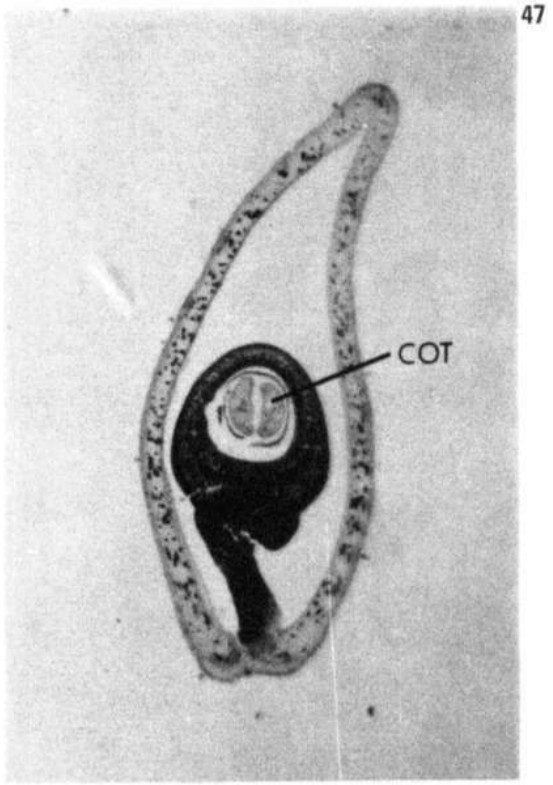
F1g. 42 : L.S. ovule, showinq embryo sac (arrow), cv. ST-1 (Haem.) x 113.

F1g. 43 : T.S. 8-day-old pod wall showing glandular hairs and secretory ducts, cv. ST-1 (Saf. - A.B.) x 75.

Fig. 44 : Surface of fresh pod wall showing spherical oil-filled glandular hairs, cv. NP(WR)-15 x 18.







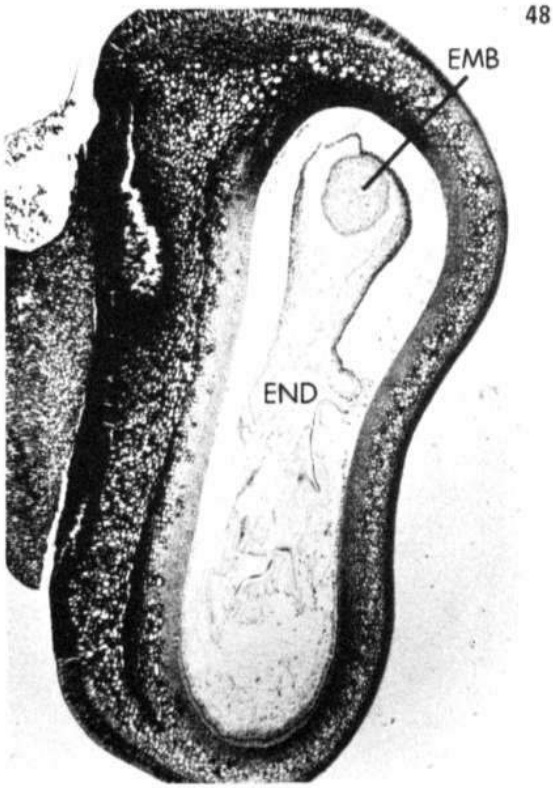
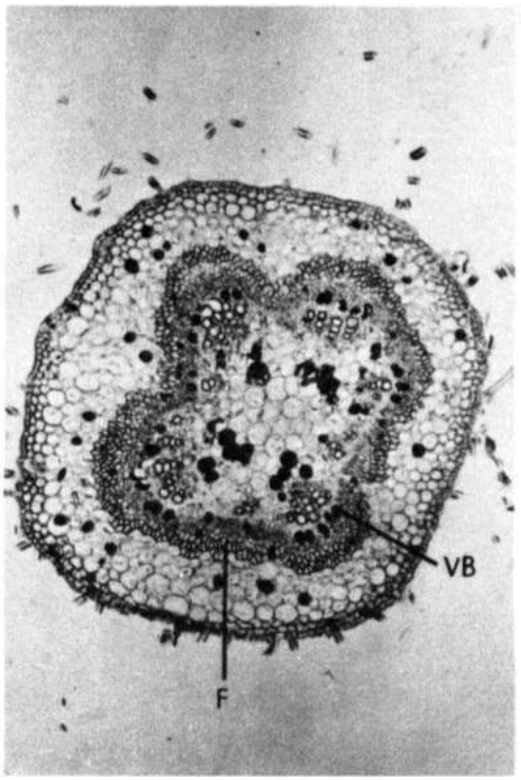


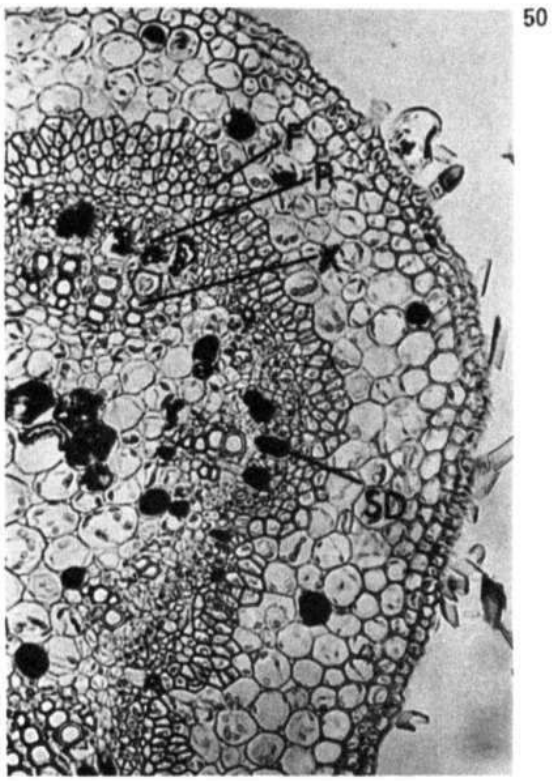
Fig. 45 : T.S. 7-day-old pod, showing the endosperm within the developing seed, cv. ST-1 (Saf. - A.B.) x 30.

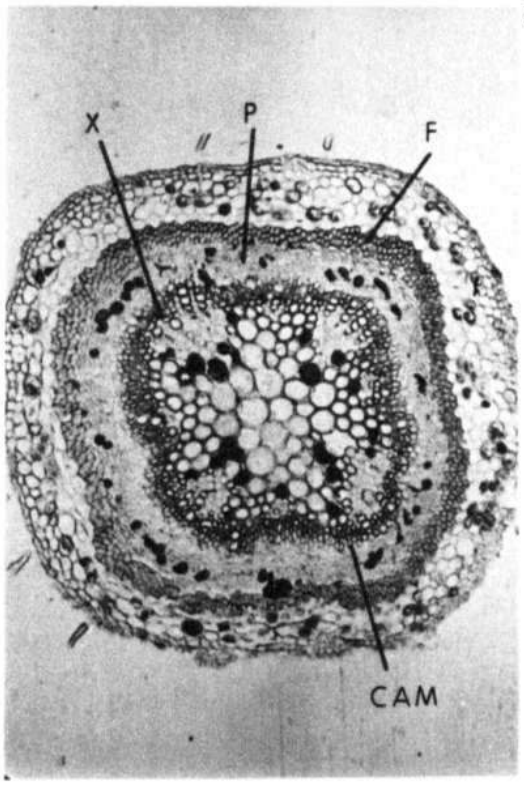
F1g. 46 : L.S. 7-day-old pod, showing the endosperm within the developing seed, cv. HY-3C (Haem.) x 38.

F1g. 47 : T.S. 14-day-old pod, showing distinct cotyledons within the developing seed, cv. ST-1 (Saf. - Haem.) x 15.

Fig. 48. : L.S. 14-day-old developing seed showing endospermous tissue and part of the embryo, cv. ST-1 (Haem.) x 30.







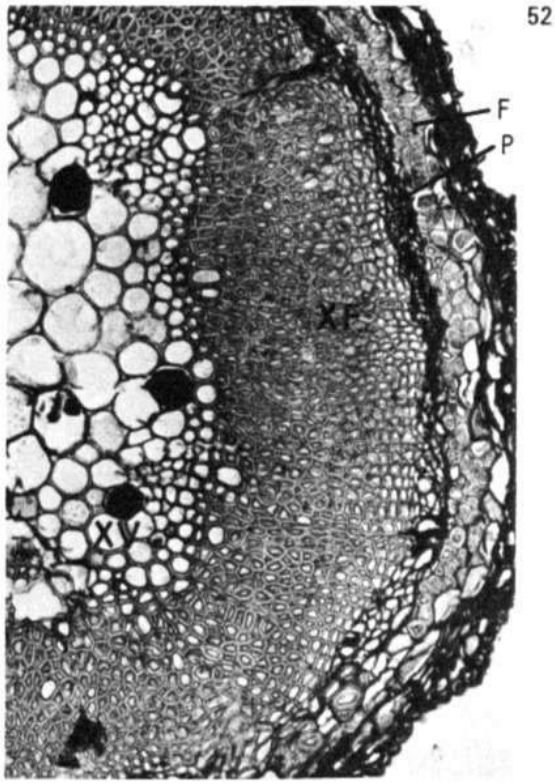
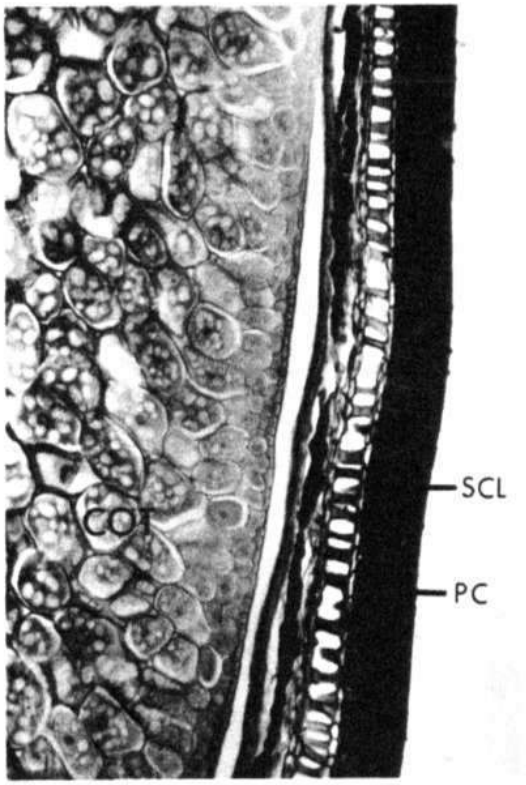


Fig. 49 : T.S. pedicel of flower, showing vascular bundles surrounded by a ring of fibers, cv. ST-1 (Haem.) x 30.

Fig. 50 : T.S. pedicel of flower, showing primary vascular tissue, cv. ST-1 (Haem.) x 113.

Fig. 51 : T.S. pedicel of 8-day-old pod, showing early stages of secondary thickening, cv. ST-1 (Saf. - A.B.) x 30.

Fig. 52 : T.S. pedicel of 49-day-old mature pod, showing secondary thickening, cv. ST-1 (Haem.) x 94.



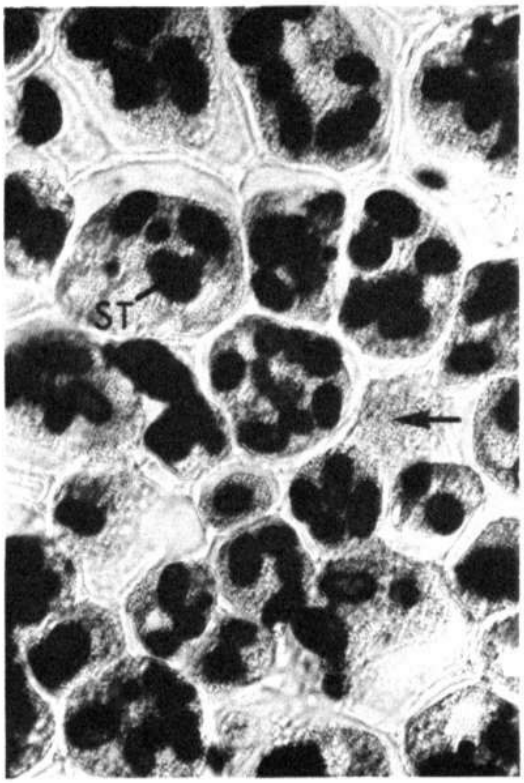


Fig. 53 : T.S. seed at end opposite the hilum, showing the seed coat and the cotyledons, cv. T-21 (Tol. B.) x 45.

Fig. 54 : T.S. seed coat showing outer layer of sclereids and inner layer of pillar cells, cv. T-21 (Tol. B.) x 113.

Fig. 55 : T.S. seed in hilum region showing funicular tissue, the opening in the seed coat and the tracheid island (arrow), cv. T-21 (Tol. B.) x 75.

Fig. 56 : L.S. seed showing starch grains and protein bodies (arrow) in cells of the cotyledon, cv. T-21 (stained with iodine and picric acid) x 300.