RESULTS AND OBSERVATIONS

3 RESULTS AND OBSERVATIONS

3.1 Crataeva nurvala Buch.Ham. (Capparaceae)

3.1.1 General morphology

Crataeva nurvala Buch. Ham.(Capparaceae) commonly known as Varuna, is a medium sized, unarmed deciduous tree with ash coloured bark. The leaves are trifoliolate and exstipulate. The tree produces new foliage twice during a year i.e. in August/September and March/April (figs. 1 and 2). According to the classification of Longmann and Jenik (1974) based on the leaf retention and shedding in tropical forest trees, this deciduous tree shows periodic growth. Leaf shedding occurs well before bud opening and the life span of leaves is about 5-7 months. The entire tree is leafless for several weeks. Leaf shedding and bud opening do not appear to be related.

The trifoliolate leaves are petiolate and petiolulate. The length and width of leaflet vary from 9-15 cm and 8-10 cm respectively. All the leaves are clustered at the end of branchlets (Shah, 1978).

3.1.2 Sequential elongation of the petiole

Sequential development of the petiole has been studied in order to examine the development of procambium, cambium

and phloem. Each petiole elongates slowly, accelerates to a maximum rate of elongation and then undergoes a progressive decline in growth rate. When the length of the petiole is graphed against time the resulting curve corresponds to the classical growth curve (fig.3).

A mature petiole measures about 11-15 cm. The petiole, which was 1 cm long when marked initially, ceased to elongate within 10-14 days. Of the three regions marked equally in the young petiole, the basal region elongates more and stopped to elongate after the cessation of elongation of the middle and distal regions. The middle and distal region fully elongates within 8-10 days with the middle region elongating more than the distal region. The basal region ceased to elongate at the twelfth or thirteenth day of marking. Petiolules elongated at a slower rate than the petiole and became 1 cm long at the eighth or ninth day. Expansion of the lamina continued even after complete elongation of the petiole.

3.1.3 Internode-node-petiole continuum

3.1.3.1 Nodal anatomy

The young internode of Crataeva nurvala shows about 72 vascular strands. Most of them are collateral and a few are phloic. Cortical or medullary bundles are absent. The

leaves are arranged in a 2/5 phyllotaxy. The leaf at the seventh node from the shoot apex is vascularized by about 14 strands. Fourteen bundles of the internode diverge towards the leaf near the node (fig.4).The node is multitrace, unilacunar (fig.5). The leaf trace strands diverge in the internode far below the leaf scar area and traverse upward in the cortex. They are arranged in a shallow arc (fig.6). A few strands lying at the abaxial region divide. Vasculature of the petiole at the basal region in a transection is ring-like with an adaxial opening (fig.7). As the bundles traverse the petiole, they frequently divide into two or more discrete strands (fig.13), each separated from one another by interfascicular parenchyma.

3.1.3.2 Young petiole vasculature

There are about 27 vascular strands in the basal region of the petiole. The open ring-form of the vasculature gradually becomes a closed one at the distal region. Petiole at the distal region is adaxially compressed and the vascular strands at the two lateral sides are elongated when compared to those of the basal region. The vascular strands are encircled by a starch sheath (fig.8). The division and amalgam of strands continue as they extend towards the petiole-petiolule junction. There are 29 strands at the middle region and 36

strands at the distal region. When the vascular system trifurcates into three petiolules the number increases and 42 strands are observed.

As mentioned earlier, there are 27 vascular strands in the basal region of the petiole. The collateral strands vary in size. Large bundles are the median ones lying at the abaxial region of the petiole. Although the branching and amalgam of vascular strands occur even at short distances, the noticeable deviation is prominent only in the middle region.

In the basal region of the petiole (fig.15) four strands end blindly. They are 2A4, 7A1, 7A4, and 12A3. The phloic strand 1A1 blindly terminates in the middle region. Towards the distal region of the petiole the number of strands increases due to frequent bifurcations. During their course into three petiolules, the vascular system trifurcates. Vascular strands from 1A4 to 4A6A8 + 5A3A4' form the vasculature of a lateral petiolule, 11A1 to 14A7 forms that of the second lateral petiolule. Departing from the petiole the vascular strands 1A5, 1A6, 14A8, 14A6, 14A6A8 + 5A3A4" to 10 form two inverted arcs but later they rearrange to form a shallow arc in the petiolule. Each petiolule in its middle region shows 5-7 composite bundles.

During their further course into the midrib the number of strands decrease due to the lateral divergence of vein traces.

3.1.3.3 Vascular architecture in a fully elongated petiole

The leaf studied was at the fourteenth node from the shoot tip in which the primary vascular system has been fully established in its petiole. The nature of the parastichy in Crataeva is such that petiole vascular bundles are organized from about 14 bundles of the internode (on the side of the leaf) (fig.14). This explains why the dye safranin mainly accumulates at the petiole side of the internode part in the IL-system (fig.16). When applied to the PL1-system in the basal splits, the dye sucked up via the main bundles. Hand sections of the petiole at different levels gives the clue of vascular interconnection in the petiole when the dye traverses from basal to the distal region and then to the petiolule (fig.17). Safranin taken up by one of the three splits of PL1-system is distributed over one of the leaflets at the feeding side. Hand sections of petiole and petiolule showed that the dye moved through the lateral vascular bundles directly connecting the area of application and stained leaflet. Application of safranin, fast green and toluidine blue at the three basal splits revealed the same pattern.

However, in some cases, unilaterally applied dye spreads via lateral connections to other vascular strands in the petiole, probably due to unbalanced water supply.

Measurements made at different levels of leaf trace strands in the petiole show that their average transverse diameter progressively decreases at higher levels whereas the number increases from 35 at the basal to 40 at the distal region (fig.18).

3.1.3.4 Anatomy of the mature petiole

The vascular system in this petiole consists of a system of strands (= vascular bundles). The collateral strands extend along the petiole as an interconnected system which forms a continuous cylinder around the central pith. Leaf used in the present study was obtained from the sixteenth node from the shoot tip. At this node about 10 bundles diverge from the central cylinder of the internode and extend across the cortex into the petiole (figs.19-24). Node is multitrace, unilacunar. Where the vascular bundles diverge into the petiole, the region of the vascular ring that confronts it, through which it would have extended had it not diverged, is occupied by parenchyma, the leaf gap. Thus, the petiole vascular system organizes from the 10 vascular bundles of the internode.

Epidermis is the outer layer, which consists of a single layer of barrel shaped or radially elongated compact cells having no intercellular spaces among them. The outer walls of the epidermal cells are cuticularised (fig.9). A multilayered hypodermis of collenchyma cells is found immediately beneath the epidermis. This collenchyma make the supporting tissue of the petiole. Thin walled parenchyma cells having well defined intercellular spaces constitute the ground tissue. The vascular bundles are arranged in a complete ring in the ground tissue.

The arrangement of vascular bundles in the petiole varies mostly in distal region and pulvinus base. In the pulvinus base they are widely separated and arranged in a ring. In the distal region they are closely arranged with a prominent adaxial flattening and marked elongation. There are 31, 33 and 35 strands in the basal, middle and distal regions respectively.

All the vascular strands are collateral. The endodermis or starch sheath is absent. In the pulvinus base and distal region a group of non-lignified collenchymatous tissue cap the vascular strands (figs.10 and 12). In the middle region these cells progressively differentiates as fibre cells. They constitute protophloem fibres (fig.11).

3.1.4 Architecture of the leaf

The basic axis of orientation of the middle leaflet is apical. The curvature of the leaf elements is concave i.e. curved toward the centre of the leaf. Leaf organization is compound; leaf is divided into separate laminar subunits. The laminar subunits are attached at the apex of the petiole, palmately trifoliolate. The petiole does not extend beyond the point of attachment of leaflets but ends in a somewhat swollen apex to which leaflets are attached by petiolules. The lamina of the middle leaflet is symmetrical (fig.25) whereas in lateral leaflets only base is asymmetrical (fig.26). Lateral leaflets are obovate, strongly obliquely faclcate, middle one is elliptic lanceolate, equilateral. Apex of the leaflets is acute, margins markedly concave, long acuminate. Base of the leaflets is acute and decurrent, margins forming an angle of less than 90° and extending downward along the petiole at a gradually decreasing angle to it. Leaf margin is entire, forming smooth arc without any noticeable projection or indentations. Leaf texture is chartaceous and opaque. The petiole bears colletors, acropetiolar. The petiole is inflated and petiolule is normal. Venation of the leaflet is pinnate with a single primary vein (mid vein) serving as the origin for higher order venation. Venation is brochidodromous. Size of the primary vein is moderate and its course is straight. Divergence of the

secondaries from the mid vein is at an acute angle (45°-65°). Upper secondary veins are more acute than lower ones. The relative thickness of the secondary veins is moderate, their course is uniformly curved. Intersecondary veins are simple consisting of a single vein segment. Angle of divergence of tertiary veins is acute obtuse or right obtuse. Tertiary veins anastomosing with other tertiary veins or with secondary veins form a reticulate, angles of anastomoses vary. Resolution of the higher order venation is distinct (fig.27). Quarternary vein size is thin. Its course is relatively randomly oriented. Quintenary vein size is thin and its course is random. Ultimate venation of the margin is looped, the major portion of the marginal ultimate venation recurved to form loops (fig.28). Veinlets are branched once, twice or more (fig.29). Areole development is imperfect with meshes of irregular shape, more or less variable in size. Arrangement of areoles is random, their shape is irregular and size is medium (fig.30).

Plate I

Crataeva nurvala Buch.Ham. (Capparaceae) in the natural environment.

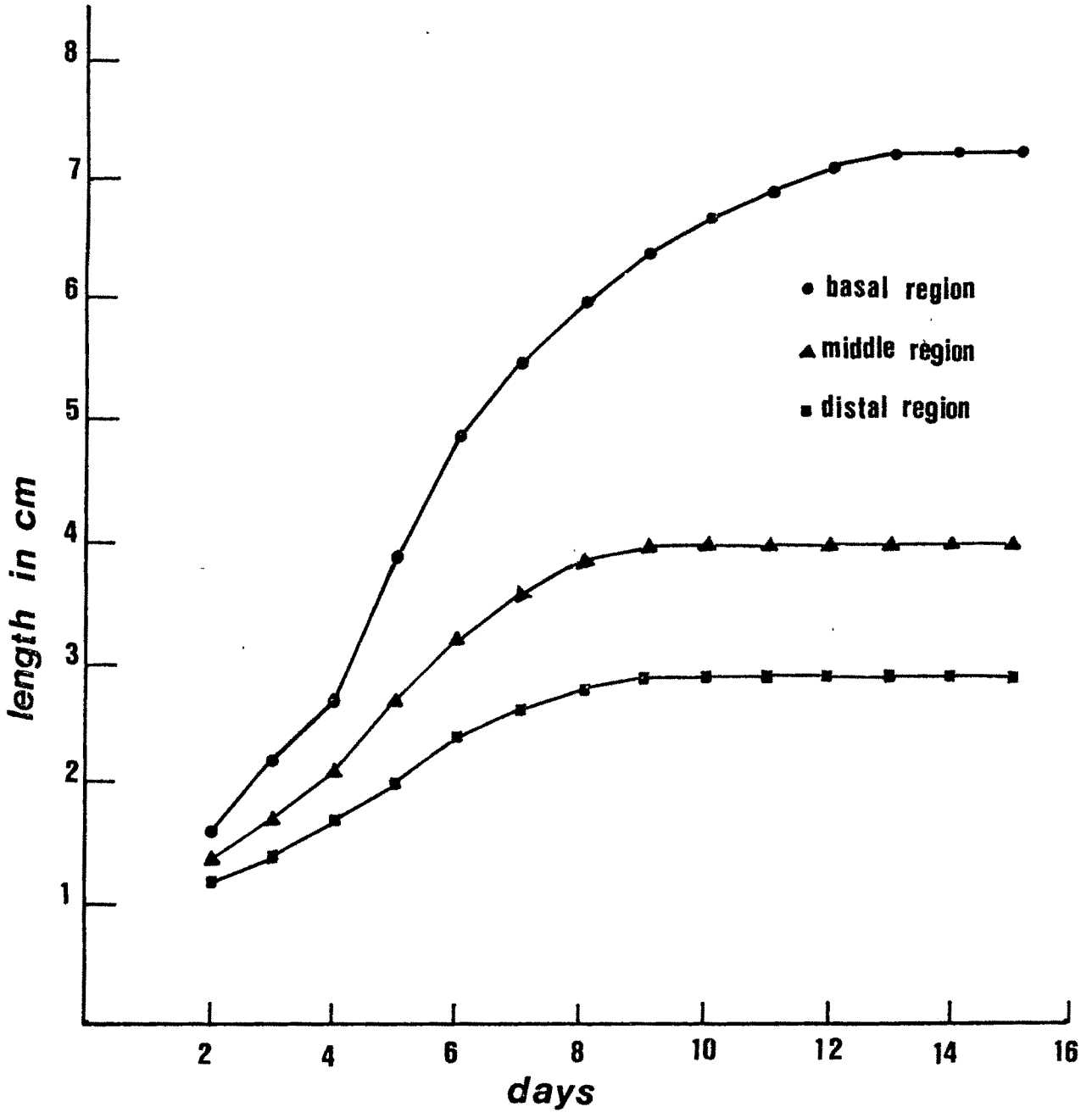
Fig. 1. Bears foliage in the month of April/May.

Fig. 2. Dormancy period in September.


①


②

Figure 3. Graph showing sequential elongation of the petiole against time.


8
7
6
5
4
3
2
1
length in cm
• basal region
▲ middle region
■ distal region
2
4
6
8
10
12
14
16
days

Figs. 4-7. Transection of the seventh node from the shoot apex.

Figs. 4 and 5. Many trace strands depart the internodal vasculature.

Figs. 6 and 7 Vascular strands completely separate from the internodal vasculature and forms the petiolar strands leaving a single gap. Bar = 100 μm.

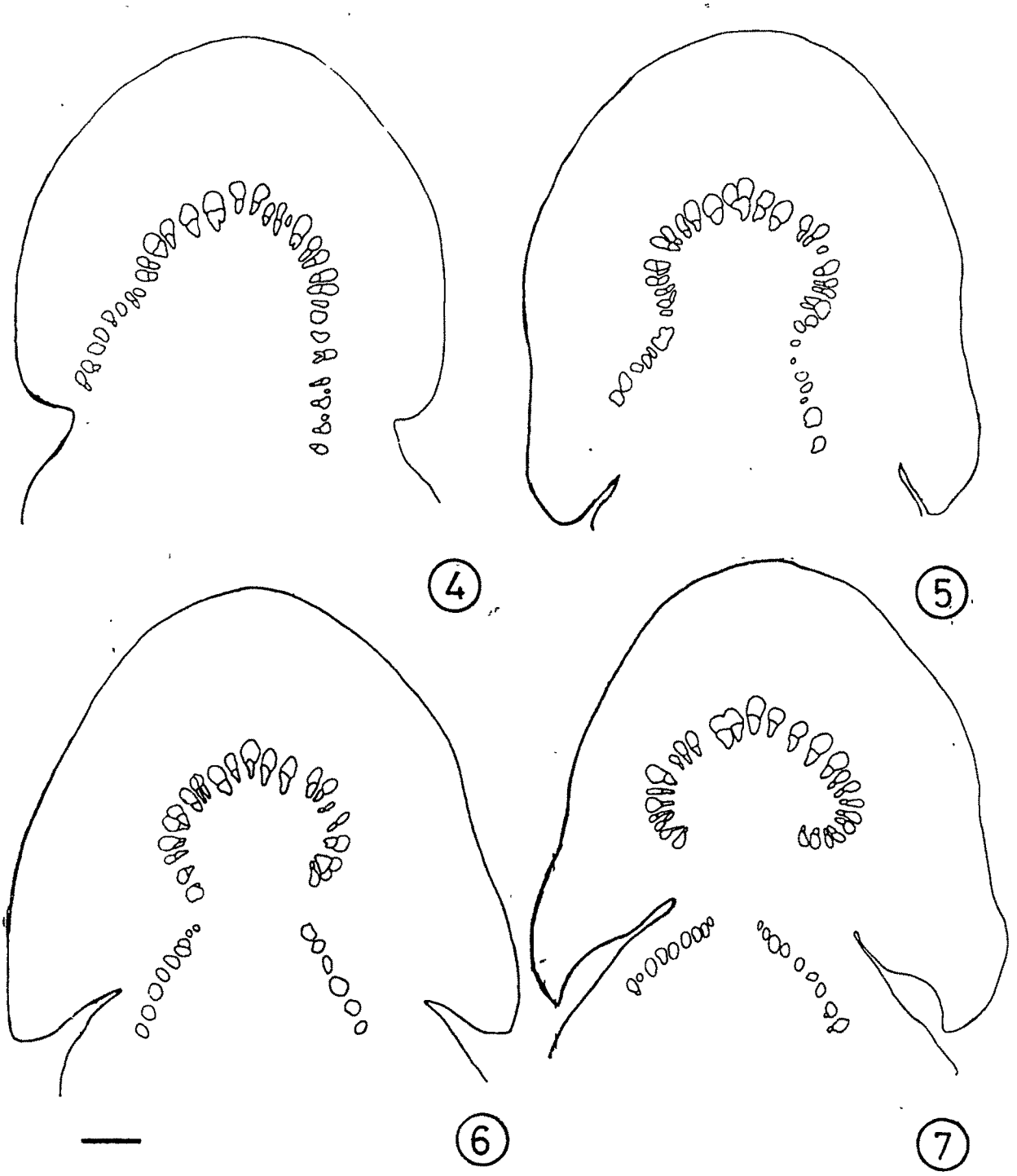

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Plate II

Figs. 8-12. Transections of petiole. A portion is enlarged.

Fig. 8. Young petiole shows starch sheath(s) on the periphery of the vascular bundles.x60.

Fig. 9. Shows the general histology of the petiole. x87.5.

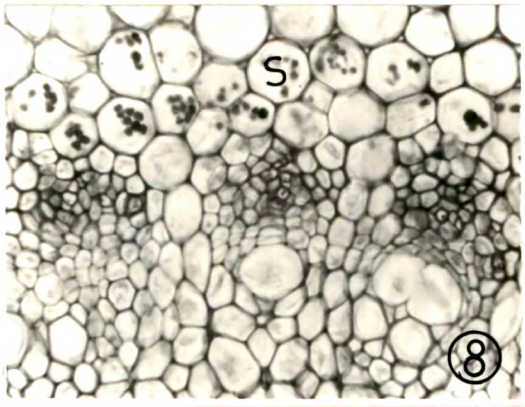
Fig.10. Vascular bundle in the basal region. x320.

Fig.11. Vascular bundles in the middle region.x62.5.

Fig.12. Vascular bundles in the distal region.x62.5. Note the collenchymatous fibre cells in figures 10 and 12 and sclerenchymatous fibres in 11.

Fig.13. A portion of the cleared petiole showing discrete vascular strands.x11.

Fig.14. Cleared internode with a portion of the petiole. Note the densely stained leaf trace strands in the internodal region.x8.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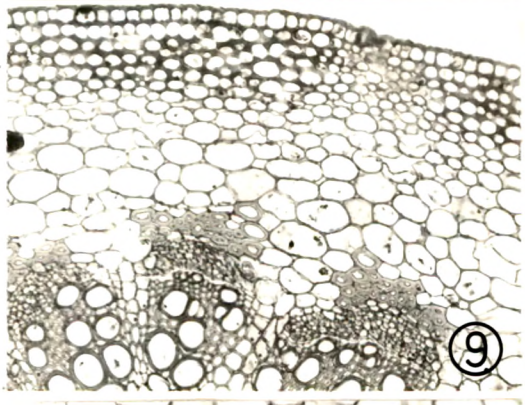
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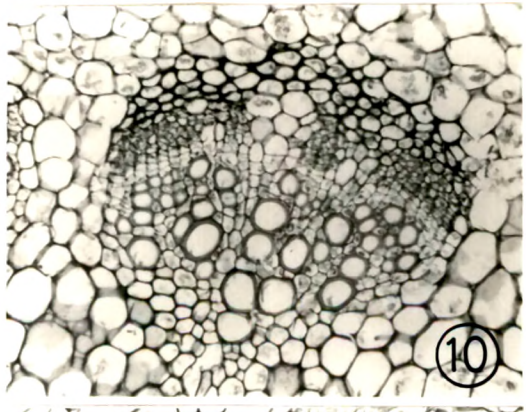
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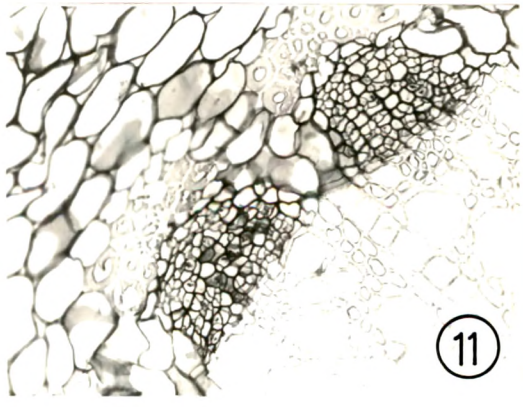
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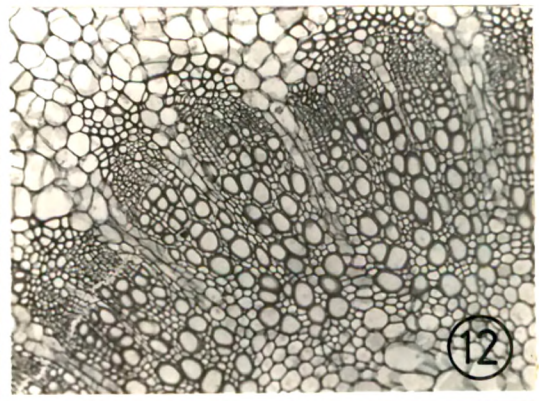
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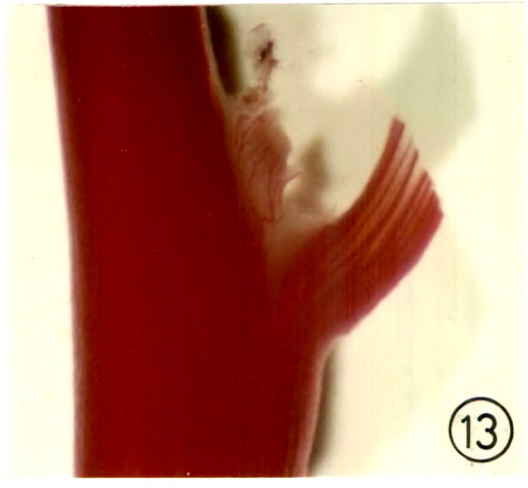
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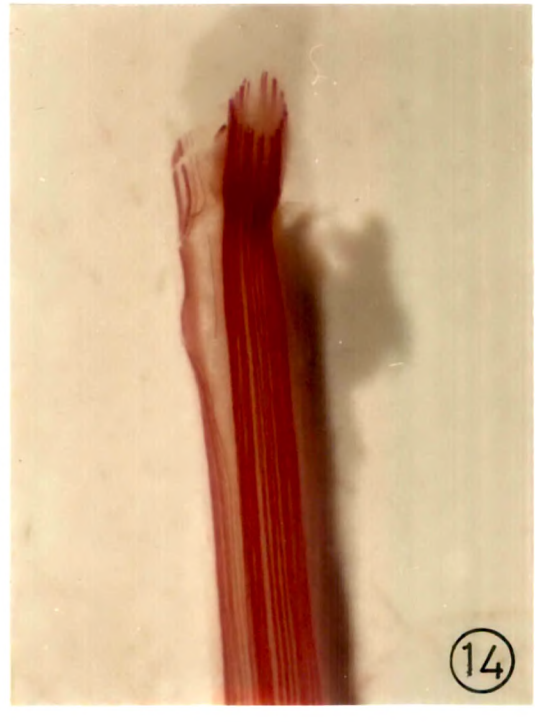
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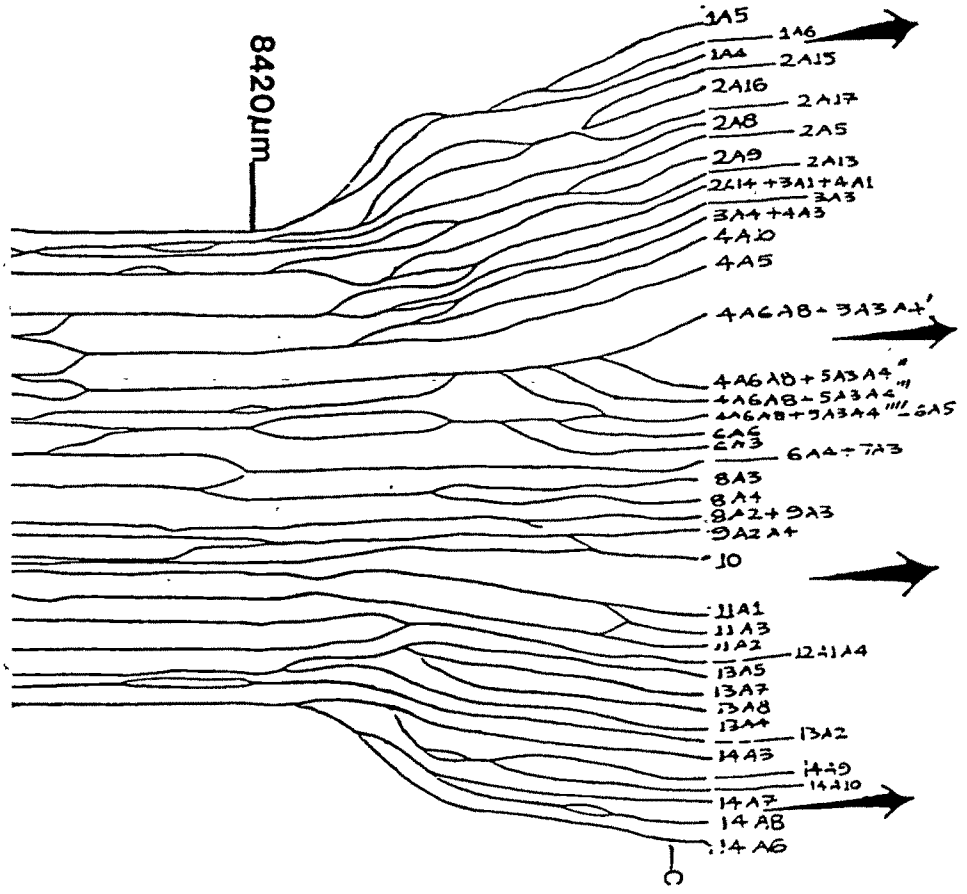
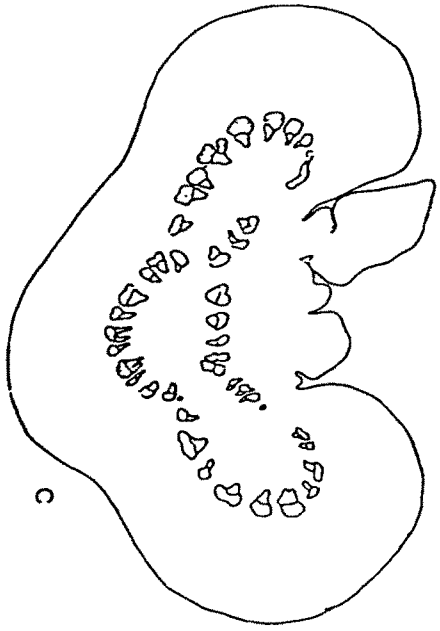
Figure 15. Diagrammatic representation of the internode-node-petiole - petiolule continuum constructed from the study of seriate sections. Measurements in um indicate the approximate length from the base. Transections A, B and C depict the arrangement of strands at the respective regions.

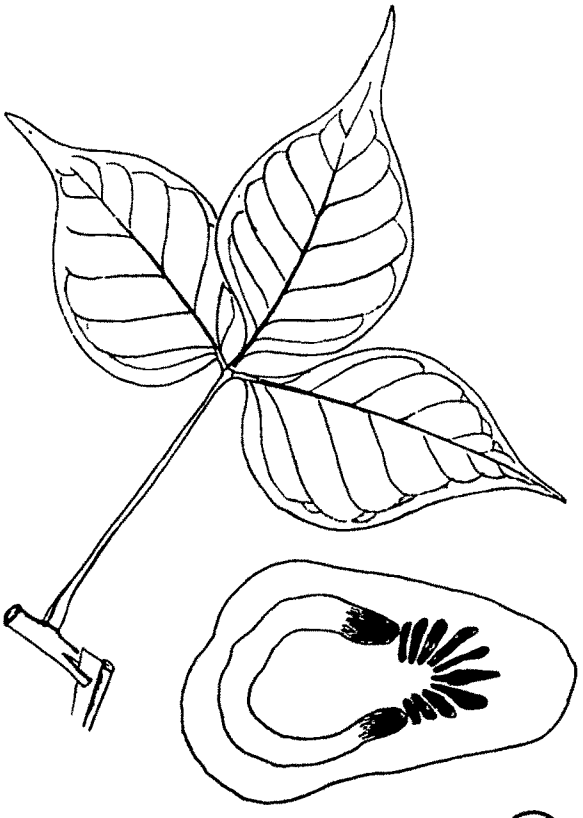

8420µm
1A5
1A6
1A4
2A15
2A16
2A17
2A8
2A5
2A9
2A13
2A14+3A1+4A1
3A3
3A4+4A3
4A10
4A5
4A6A8+5A3A4'
4A6A8+5A3A4''
4A6A8+5A3A4'''
4A6A8+5A3A4''''
6A5
6A3
6A4+7A3
8A3
8A4
8A2+9A3
9A2A4
10
11A1
11A3
11A2
12+14
13A5
13A7
13A8
13A4
13A2
14A3
14A9
14A10
14A7
14A8
14A6
C


C

Figs. 16 and 17. Dye distribution pattern resulting from dye uptake in the region of the petiole vascular strands.

Fig. 16. IL-System. Uptake of safranin in one of the basal splits resulted in unilateral transfer through the internode towards the petiolar bundles.

Fig. 17. PL1-system. Uptake of three dyes through the basal splits resulted in the distribution pattern as shown. (IL = Internode / Leaf system; PL1 = Petiole / leaflet system).



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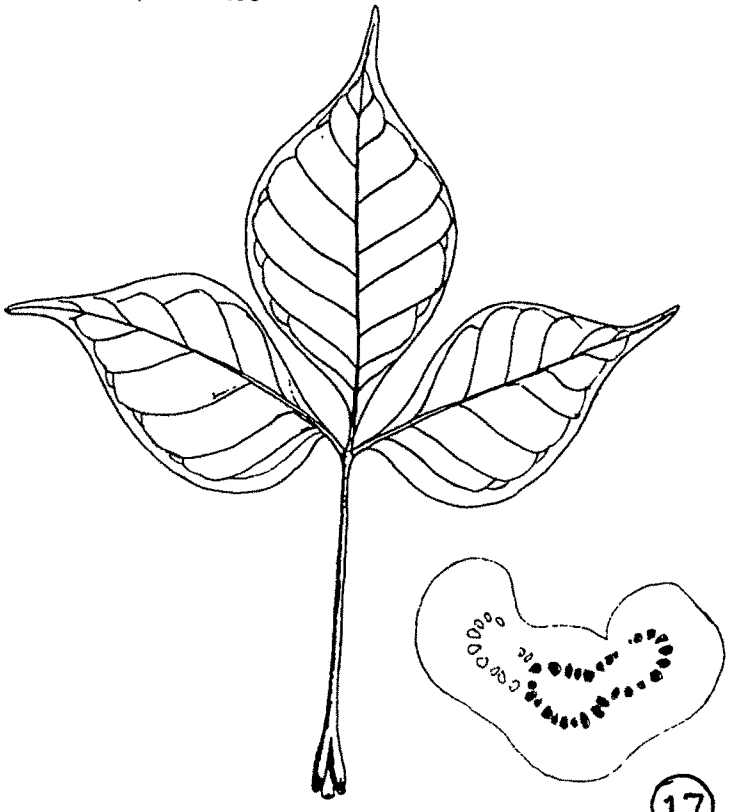

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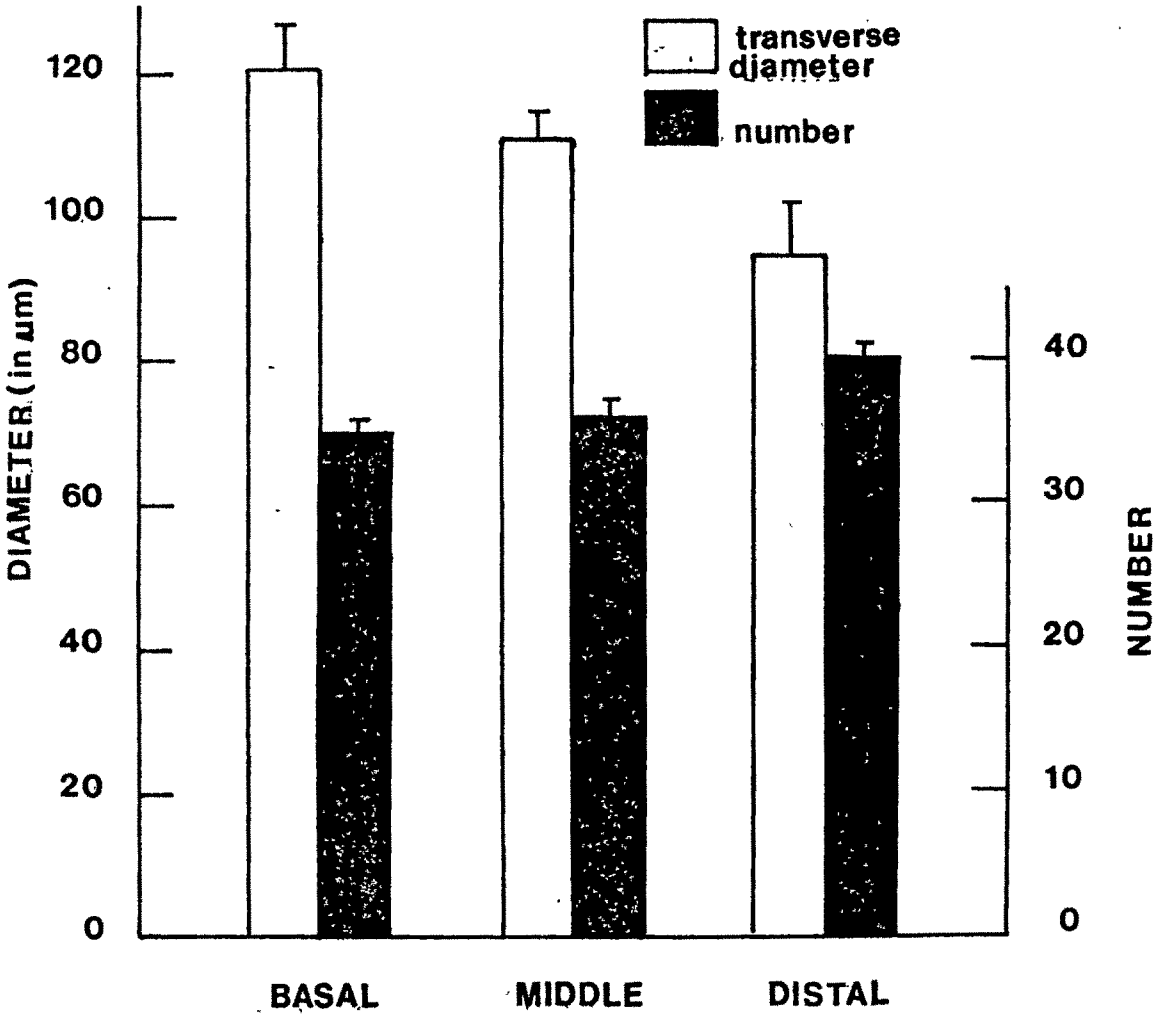
Figure 18. Average transverse diameter and number of vascular strands in the three regions of the petiole.

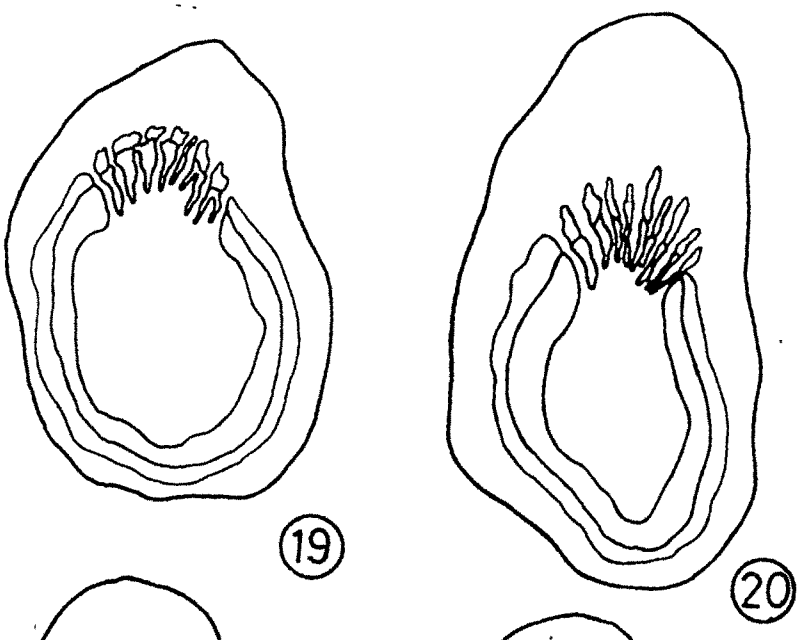

transverse djameter
number
DIAMETER (in µm)
120
100
80
60
40
20
0
40
30
20
10
0
NUMBER
BASAL
MIDDLE
DISTAL

Figs. 19-24. Transections of the mature node taken at successive levels to show the organization of petiolar strands from the internodal vasculature.

Figs. 19 and 20. Vascular strands separate from the internodal vasculature.

Figs. 21 and 22. Number of strands increase due to bifurcations.

Figs. 23 and 24. Vascular strands separates from the internode and organizes as petiolar strands. All x 15.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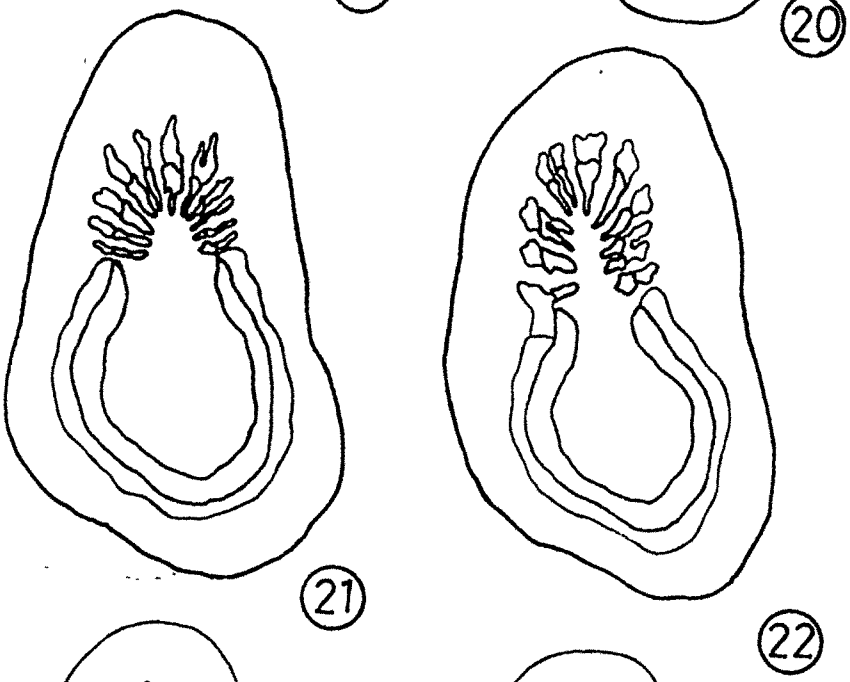
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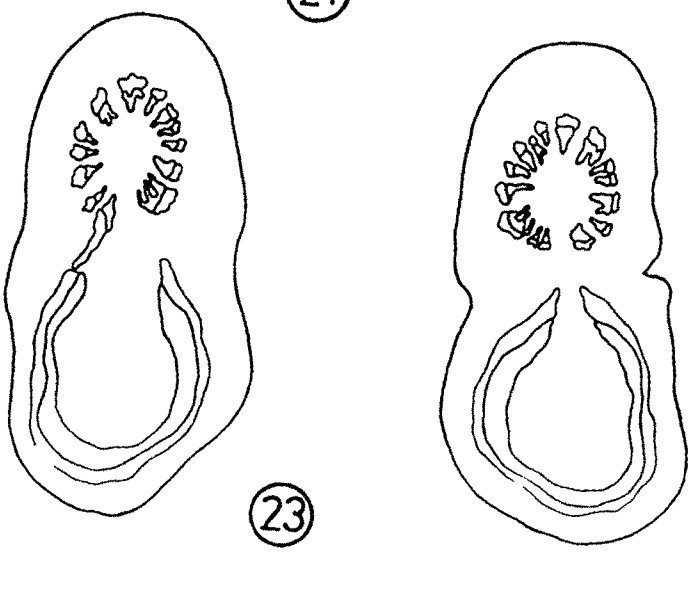
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Plate III

Figs. 25-30. Photographs of cleared lamina.

Fig. 25. Showing the vasculature of middle leaflet. Natural size.

Fig. 26. Lateral leaflet. Arabic numerals denote vein order (M = mid vein; 2' = Intersecondary veins). Natural size.

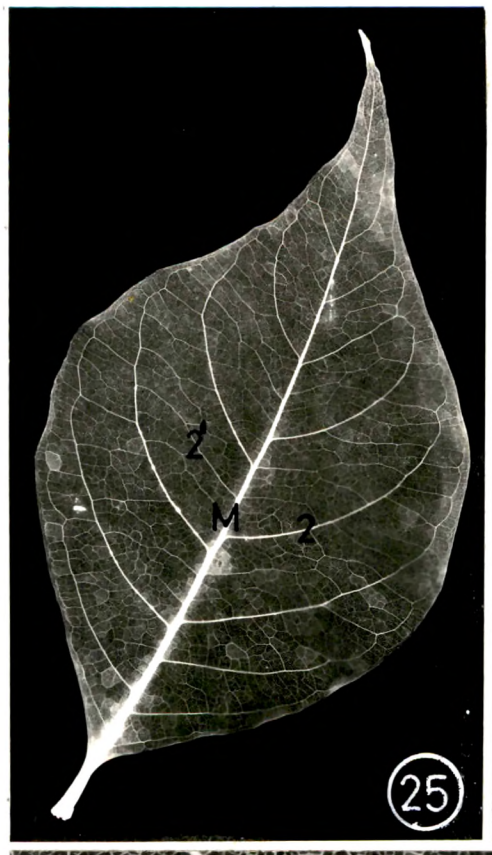
Fig. 27. Portion of a cleared lamina showing mostly higher order veins. x 6.

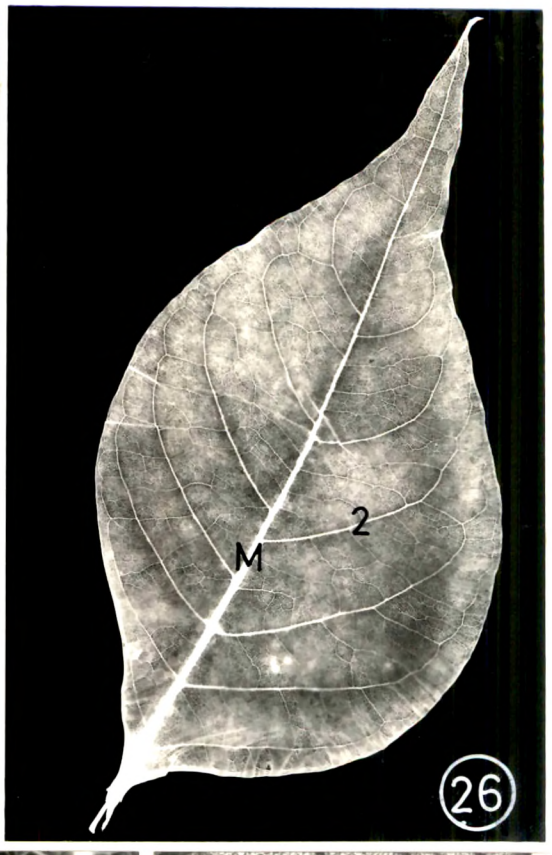
Figs. 28-30. A portion of figure 26 enlarged.

Fig. 28. Showing loop formation of higher order veins in the margin (arrow head). x 24.

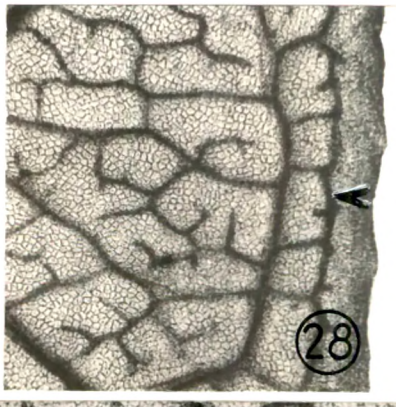
Fig. 29. Showing branched (at arrow) and unbranched (arrowhead) veinlets. x 24.

Fig. 30. Showing areoles. Note the empty areoles. x 48.


2
M
2
25


M
2
26


3
4
5
2
M
27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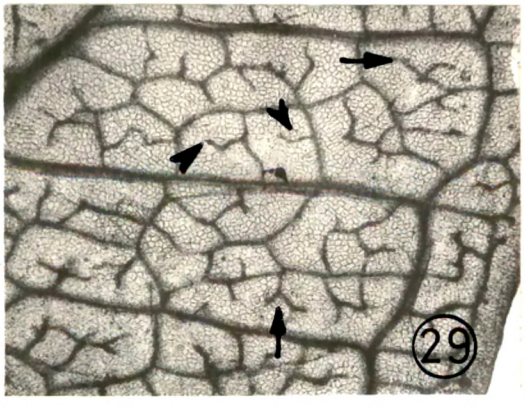
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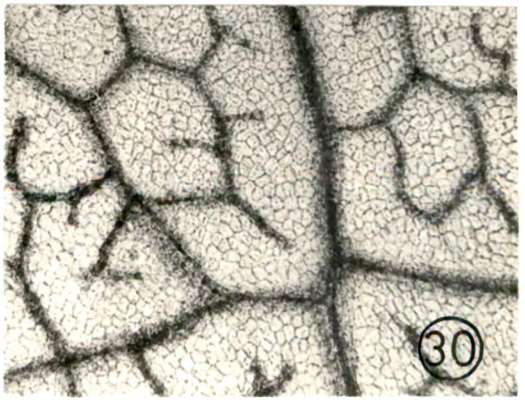
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SECTION II

3.1.5 Differentiation of procambium in the petiole

3.1.5.1 Transverse course of differentiation

To trace the course of procambium in transverse sections of the petiole, the position of successively appearing leaves is referred to the respective nodes in the shoot apex. Where the petiole is not differentiated, the observations are confined to the basal region of a leaf primordium because this region presumably develops into a petiole.

A sharp demarcation between procambium and adjacent cells is absent in the early developmental stage of the primordium at the first node. A darkly stained and actively dividing locus could be observed at the centre of the developing primordium which may be considered as the forefront of a developing procambial trace (fig.1). A recognizable developing petiole is present at the second node where three procambial strands of a unilancunar leaf trace are noticed at the basal region (figs. 2 and 3). These three strands are arranged in the form of an arc along with the intervening and surrounding vascular meristem. The entire region is the forefront of future primary vascular system of the petiole/leaf. The term

vascular meristem is being used here to distinguish the other vascular meristem cells which cannot be distinguished as procambium, but its relationship with apical meristem is not in anyway considered here as it is considered when the origin of primary vascular system in the stem axis is investigated. This meristematic region along with the procambial strands is the specific blocked out region in the ground tissue identified on the basis of cytoplasmic density and cell orientation. At the basal region the median strand is more differentiated than the lateral ones showing both protoxylem and protophloem differentiation.

Basal region of the leaf at the third node shows a vascular arc with six procambial strands (fig.4). Towards the distal region of the petiole the discreteness of these strands is not observed but the arc of vascular meristem is visible. All these procambial strands do not develop simultaneously. The differentiation is marked especially at three loci, median and laterals where ultimately petiolule develops. Basal region of the petiole at the fourth node shows eight procambial strands arranged in a shallow arc interspersed with the vascular meristem (fig.5). In the distal region of the petiole the discreteness of these procambial strands is less distinct and a darkly stained vascular meristem is observed in

which certain loci appear to be more differentiated; these are centres of procambial differentiation. These procambial strands constitute a unilacunar leaf trace with many strands. Considering the trend of procambial development in the differentiation of primary vascular elements, the acropetal development of the procambial strands is confirmed. The indication of the presence of a procambial strand is the deep staining of a small group of irregularly oriented and actively dividing cells arranged in different loci, each separated by interfascicular vascular meristem. The conspicuous aggregation of cell mass at different loci is evident in figure 8. The development of the procambium is accompanied by gradual parenchymatization of the prospective pith and cortex.

3.1.5.2 Longitudinal course of differentiation

The shoot apex of Crataeva has a tunica - corpus organization with a single layered tunica overlying the corpus. The apex shows cytohistological zonation; showing central meristem, peripheral meristem and pith meristem (fig.6). The cells of the tunica as well as those of two or three outer layers of the corpus are elongated perpendicular to the curved surface of the apical dome. Vacuolation in the central ground meristem cells is

noticeably evident at 76 um from the tunica apex. Above this level the peripheral meristem appears morphologically and cytologically homogeneous. The leaf primordia originate at the peripheral meristem.

The leaf primordium at the second node shows a darkly stained central meristematic region. Longitudinally it is recognized as a strand of elongated cells (fig.7). The elongated form is attained by frequent longitudinal divisions accompanied by little lateral widening of the cells. These procambial cells appear narrower than the adjacent ground meristem cells. Intervening vascular meristematic cells do not show any specific difference, whereas procambial cells elongate and become longer than the ground tissue cells which divide transversely. Intervening vascular meristematic cells differ from procambial cells only in having less affinity for staining. The leaf primordium at the third node shows longer procambial cells at the basal region. As the procambial trace differentiates and longitudinally traverses in the leaf primordium, the distal region of the strand shows procambial cells which are less elongated, less closely aggregated and somewhat lightly stained as compared with the procambial cells below. They are on their way to differentiation of a stabilized procambial stage. Longitudinal sections confirm that the

leaf trace procambial development is acropetal.

3.1.6 Procambium and the primary vascular differentiation

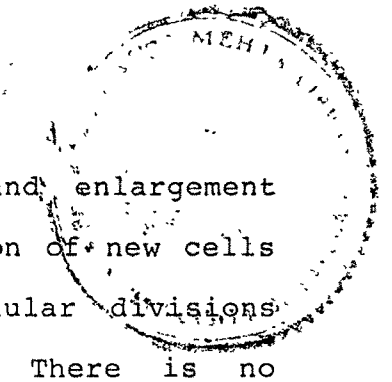
Analysis of the development of primary vascular system in the petiole is based on the study of its leaf procambial traces and their role in the formation of the vascular system. Procambium is identified according to the criteria set forth by Esau (1965a). Primary phloem elements are identified by their position in the leaf trace strand, their relative sizes and shapes, their thick walls, lack of cell contents and presence of callose. Protoxylem elements are identified by their small size and rounded appearance, and birefringence of their walls in polarized light. Metaxylem elements are distinguished from protoxylem by their late appearance in the leaf trace, their large diameter and their relative position with regard to the protoxylem. However, it should be noted that in Crataeva the delimitation between the last formed protoxylem and first formed metaxylem is not precise.

The growing shoot of Crataeva nurvala produces petiolate leaves in an alternate fashion, each separated at maturity by a clearly defined internode. Early developmental stages of the petiole are categorized on the basis of their length into five stages.

Stage	Petiole length in cm	Approximate position from the shoot apex
I	0.3	4th node
II	1.0	7th node
III	5.6	12th node
IV	8.6	14th node
V	14.0	16th node

From observations made on petiole elongation (Section I, Chapter 3) it was found that primary growth in the first four stages and the fifth stage shows transitional or early phase of secondary growth.

As mentioned earlier the leaf trace is unilacunar, multitrace. During the development of multitrace condition at the early nodes, new procambial strands arise in the vascular meristem of the petiole completing the full development of the leaf trace. These strands are in continuity with the axial vascular system and develop acropetally. The indication of the presence of a procambial strand is the deep staining of a small group of irregularly and compactly arranged and actively dividing cells in the vascular meristem (fig.8). Procambial enlargement proceeds concomitantly with acropetal advancement resulting in a procambial strand that is considerably larger and better developed at its



base than at its advancing front. Strand enlargement occurs both by the acquisition or accretion of new cells from the vascular meristem and by cellular divisions within the procambium of the strand. There is no definite plane of division in the procambium. Although the cell divisions appear to be random, they nonetheless follow a pattern in the sense that a well organized procambial system is produced. In the petiole at the third node there is an increased vacuolation of cortical parenchyma cells, and the procambial cells are characteristically elongated longitudinally and have narrow diameter in transverse plane (fig.9). These cell dimensions, together with the dense cytoplasm demarcate the procambium from cortex and pith cells.

Protophloem develops acropetally and in continuity with older phloem below. The first protophloem elements are initiated towards the cortical side of the strand and further protophloem differentiation is centripetal. The differentiation of first protophloem elements takes place earlier than that of the protoxylem. Subsequent development of protophloem and metaphloem is described in Section III.

Concurrent with the foregoing processes, some procambial cells between phloic and xylary regions divide periclinally and, as a result, procambial cells

progressively show radial seriation (fig.10). This marks an important phase in the development of procambium because in the earliest stage of procambial development the plane of divisions has been irregular. Subsequent development of a definite periclinal orientation of cells is a marked change in the procambial differentiation. Larson (1976 , 1982) defined the radially aligned cells resulting from the early periclinal divisions the 'initiating layer'. In the petiole at the fourth node these cells either occur in two or three tiers or as few isolated cells (fig.11). During the protoxylem and protophloem development the continuity of this layer is not complete in the strand and there is no complete segregation of protophloem and protoxylem tissue at this stage. The serially arranged procambial cells are not equidistantly placed between the two poles of the strand, rather it appears well to the inside (fig.12). All procambial tissue external to the seriated layer is considered phloic procambium and all tissue internal to it xylary procambium. It should be noted that the identity of tangentially continuous seriated layer could not be confirmed because one or more radially aligned periclinal divisions occur and therefore the formation of continuous seriated layer across the width of the strand at one stage of development is not distinct. First evidence of seriation is the appearance of isolated periclinal divisions (see fig.11). They

initiate the first recognizable radially aligned cells within a vascular strand and they may be considered precursors of the metacambium that subsequently develops (figs.13 and 14). During this development the interfascicular region differentiates as parenchyma.

3.1.6.1 Metacambial development

Although I concur with the concept that procambium and cambium are sequential stages of the same meristem, continuum can be subdivided for easy understanding. Vascular strands in a 5.6 cm long petiole show a zone of cambiform cells internal to the primary phloem (fig.15). Larson (1976 , 1982) has referred a similar zone as metacambium in Populus deltoides. The seriated band of metacambium is 3-4 cell layered. It is characterized primarily by almost regular periclinal divisions. The metacambium is a more advanced meristematic stage of the continuum in which additional periclinal divisions are interposed to develop it into a tangentially continuous band of radially aligned cells. Metacambial cells are further distinguished from the surrounding cells by their greater activity resulting into a more definitive radial and tangential alignment, and narrower radial cell diameters. Once the metacambium within the strand has attained a certain level of distinct morphological

identity in a petiole, then its derivatives differentiate as metaxylem and metaphloem. But I have some evidences to conclude that the metaxylem elements may be formed from isolated periclinal divisions of the procambial cells with no precocious indication of the formation of metacambium (see fig.27). Here, the existence of the initiating layer as defined by Larson (1976) could not be conclusively confirmed.

Metacambium is also identified in a 8.6 cm long petiole. Regardless of their stages of development almost all the collateral strands show a band of 3-5 layered metacambium (fig.16). The continuity of the metacambium may become indiscrete in some of the strands by one or two rows of radially elongated interspersed parenchyma cells (fig.17). They are the product of metacambium cells. At this stage the metacambial cells show frequent transverse and longitudinal divisions. The evidence for a longitudinal division is from the fact that phragmoplast is arranged in two zones as a 'halo' perpendicular to the longitudinal axis of the cell (figs.18 and 19). In such cells the young nuclei have a rounded appearance with one or two nucleoli, while the cell plate has not reached the end walls. Structurally the metacambium resembles with that of the previous stage of the petiole.

The fifth stage of the petiole shows transition in its vascular development or early initiation of the secondary growth. As in the transition between procambium and metacambium, there is also intergradation between metacambium and cambium.

3.1.6.2 Morphological and dimensional changes

The procambium is a homogeneous tissue in its early development (figs. 20 and 21). Its cells have large nuclei and end walls are rounded or transverse in radial view (fig.22). The cytoplasm is densely stained. Later the procambial cells elongate along with the elongation of the petiole. During the development of metacambium there is conspicuous elongation of cells. They show pointed, transverse or oblique end walls and have a non-storied arrangement (figs.23 and 24). Their cytoplasm is densely stained with a spindle, oval to round nucleus and nucleolus. Dimensional details of the procambium, metacambium and cambium are shown in Table I.

3.1.6.3 Protoxylem development

Protoxylem is first observed at the base of the petiole and subsequent differentiation is acropetal in the developing petiole. Protoxylem and protophloem

develop at two opposite poles in a strand where the procambial cells do not show any pattern of cell orientation (fig.25). The xylary procambium within which the protoxylem pole develops consists of highly vacuolated cells that appear indistinguishable from the vascular meristem. Protoxylem develop from some of the highly vacuolated xylary procambial cells (see fig.12). Once initiated the protoxylem pole develops peripherally and centrifugally. After the protoxylem pole begins to develop random percilinal divisions appear in the strands centrifugally opposite to the protoxylem pole (fig.26). Some of these cells may differentiate as metaxylem elements (figs. 27 and 28). This development signals the subsequent formation of radial file of cells and development of metacambium. Before the metacambium is distinguished the number of protoxylem elements observed is 2-4.

3.1.6.4 Metaxylem development

It is indicated earlier that the earliest metaxylem elements can be initiated from the randomly and periclinally divided procambial cells. But once the micromorphology of metacambium is distinct subsequent development of metaxylem is from its cells. Metaxylem differentiation begins immediately after the metacambium

in the strand appears at the base of the petiole. From this site metaxylem vessel elements develop acropetally into the petiole. Differentiation of late protoxylem and early metaxylem overlaps in time, but there is a continuity of proto-metaxylem elements and radial file of elements is resulted (fig.29). Because early metaxylem formation occurs during active petiole elongation many of these elements are stretched, though not obliterated. Metaxylem formation is completed with the cessation of petiole elongation.

3.1.6.5 Transition of metacambium to cambium

Initiation of secondary growth in an organ is correlated with its cessation of elongation (Esau, 1965a). Therefore, with the cessation of petiole elongation, initiation of secondary growth in it is expected. The mature petiole is defined as the one which has ceased to elongate.

During the last stage of elongation, metacambium in the petiole shows certain structural changes. The width of the periclinally dividing metacambial cells increases radially and there is a marked thickening of the radial walls. Transverse divisions also occur in the metacambium and most of them assume the fusiform form (see figs.32

and 33). The characteristic beaded appearance of the cell wall is also observed. These changes result into the formation of fascicular cambium. There is one feature which is absent in the cambium of the petiole, i.e. the absence of ray initials. Unfortunately, I could not observe during the transition stage any development at least in one region which indicates the formation of an initiating layer of the cambium. Along with the changes in the metacambium which result into the formation of fascicular cambium the other structural changes also occur in the vascular region which are associated with the beginning of secondary growth. They are lignification of interfascicular prenchyma, xylem parenchyma, phloem fibres, formation of short tracheary elements with scalariform pitting and development of a few xylem fibres (figs. 30 and 31). Secondary phloem elements begin to differentiate from cells formed by successive periclinal divisions and plane of neighbouring cells do correspond with that of fusiform initials. Because lignified xylem parenchyma and xylem fibres are associated with secondary vessels, the primary - secondary transition was judged to occur when xylem parenchyma and xylem fibres with birefringent walls were first detected both within and between adjacent strands forming the vascular system (figs.34 and 35). Although protophloem fibres also showed birefringence, detection of lignified xylem

parenchyma and xylem fibres was consistent to confirm the metacambium - cambium transition. During subsequent development due to the lignification of xylem parenchyma the vascular strands unite to form a cylinder in which the complete identity of strands becomes less evident.

The secondary growth is confined to the vascular strands and no interfascicular cambium is observed. The fascicular cambium is 3-4 layered with radial alignment of cells (figs. 36 and 37). It consists of fusiform initials which are long, radially flattened and tangentially tapered with walls having beaded appearance (figs. 37 and 38). The fusiform initials are uninucleate, rarely binucleate and with a nucleolus. The fusiform initials give rise to all the cells of the xylem and phloem that are arranged in their long axis of the petiole. In otherwords, they form only the longitudinal system of phloem and xylem since rays are absent. The cambium is non-storied.

3.1.7 Activity of cambium in the petiole

In the petiole of Crataeva nurvala secondary phloem appears in radially arranged complexes. Derivatives of the cambial initials divide periclinally and anticlinally to form a complex of sieve elements, companion cells and phloem parenchyma. The sequence and plane of divisions of

the phloic cambial derivatives are examined to understand the ontogenetic relationship of the secondary phloem elements. This is facilitated by the analysis of radial file of cells derived from the fusiform initial. Since the radial alignment of cells is visible in transections, serial sections are examined. The number and orientation of cell divisions occurring in a derivative cell leading to the formation of a sieve tube element and its associates to form a complex may vary. In one type the derivative cell divides periclinally to form a proximal parenchyma cell and a distal sieve tube element precursor which divides anticlinally to form a sieve tube element and a companion cell (fig.40).

In another pattern the derivative divides anticlinally to form a sieve tube element and a companion cell. Two or three files of sieve tube elements and companion cells are arranged in a radial row followed by a parenchyma cell. These sieve elements do not have ontogenetically related parenchyma cells.

In certain complexes the planes of cell division are so oblique that arrangement of phloem complexes appears irregular hence difficult for analytical interpretation.

3.1.8 Vascular cambium in the stem

Vascular cambium in the stem of Crataeva is non-storied comprising two systems of cells; the axially elongated fusiform initials and the shorter ray initials being responsible for the production of horizontal ray system (fig.41). The fusiform initials are mostly uninucleate but multinucleate condition is also observed. In a transverse section of the bark the cambial zone consists of 6-9 layers of radially aligned cells (see figs. 41 and 42). Average length and width of fusiform initials is 200.81 um and 20.54 um respectively. The average radial diameter of fusiform initials is 6.10 um. The rays are multiseriate (fig.43). They are 1-6 cells wide and 2-48 cells or rarely more in height.

3.1.9 Behaviour of cambium in the petiole after wounding

The mature petiole is wounded along a length of 3-4 mm After about seven days period, there was restoration of the connection between the original wounded vascular tissues and the regenerated vascular system forming a callus (fig.46). Vascular continuity is established between the two adjacent strands. The parenchyma cells of the cortex become meristematic and undergo one or more divisions before sieve tube element differentiation is

initiated. In the case of regenerating xylem, the parenchyma differentiates directly into a tracheary element without undergoing any further division (fig.44). Analysis of the longitudinal sections from the wound callus exhibited an unusual feature. In a mature petiole of Crataeva the cambium consists of only fusiform initials. But after a wound is formed and during the healing process ray cells are observed among the population of fusiform cells (fig.45). Although the exact reason for this is imperceptible, presumably this is due to the hormonal imbalance (see Chapter 4, Discussion).

A comparison of the vascular cambium in the petiole and stem is shown in Table II.

Table I : Changes in the dimension of procambium, metacambium and cambium during the elongation of the petiole observations are confined to the middle region of the petiole (value in the brackets shows the range)

Length of the petiole in cm	Length in μm			Width in μm		
	Procambium	Metacambium	Cambium	Procambium	Metacambium	Cambium
0.3	29.64 (23.40-35.88)			4.24 (3.12-6.24)		
1.0	44.54 (25.12-72.22)			4.84 (3.12-7.85)		
5.6		113.90 (66.30-189.30)			11.90 (9.70-15.60)	
8.6		94.44 (54.95-135.02)			10.04 (9.42-10.90)	
11.6		110.13 (70.65-175.84)			11.47 (9.42-15.6)	
14.0			118.90 (48.67-141.30)			13.33 (7.85-14.13)

Table II. A Comparison of the Vascular Cambium in the petiole and stem (both July collections). Dimensions in μm

	Petiole	Stem
Cell types	Fusiform initials	Fusiform initials and ray initials
Fusiform initials		
Length	104.04	200.81
Breadth	11.96	20.54
Radial diameter	5.25	6.10
No.of nucleus	One,rarely two	One or more
Shape of the nucleus	Round, oval or spindle	Oval or spindle
No.of layers	3-4	6 - 9
Interior fascicular cambium	Absent	Present

Plate IV

Figs.1-5. Transections

Fig. 1. Basal region of the leaf primordium at the first node. x 120.

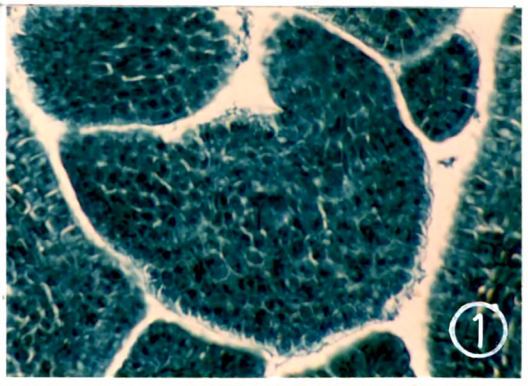
Fig. 2. Section from 90 um below tip of apical meristem showing leaf traces to the three respective (arabic numerals) primordia. x 120.

Fig. 3. Part of the section in figure 2 enlarged to show three procambial strands (arrows) in the vascular meristem which are related to the second node. x 224.

Fig. 4. Basal region of the petiole at the 3rd node (arrow heads point to the strands). x 140.

Fig. 5. Basal region of the petiole at the 4th node (arrows point to the strands). x 140.

Fig. 6. Longitudinal section of the shoot apex showing the three meristematic zones. x 171.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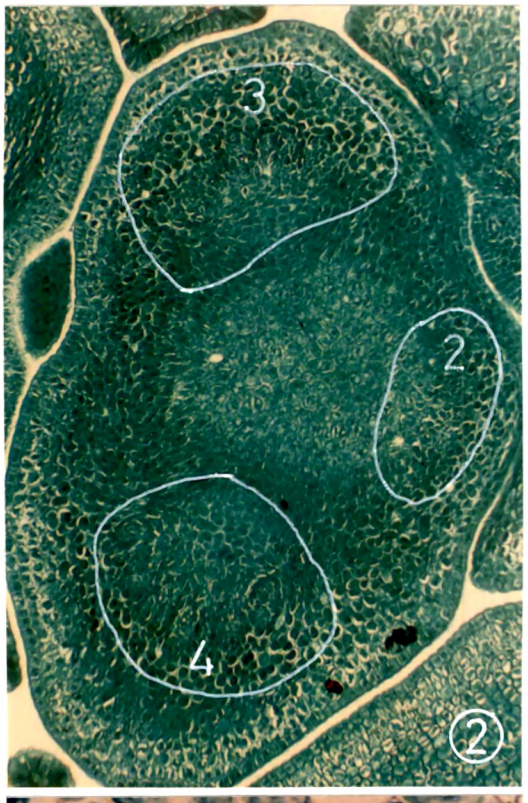
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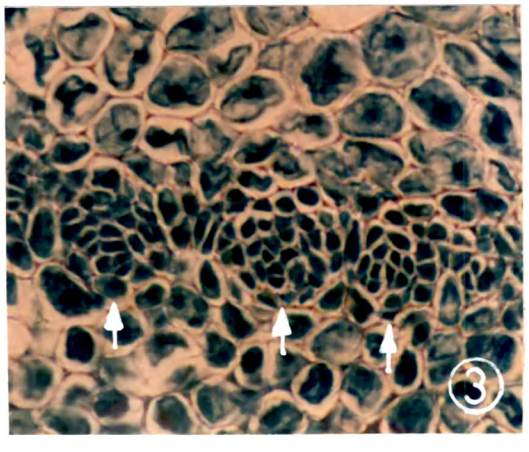
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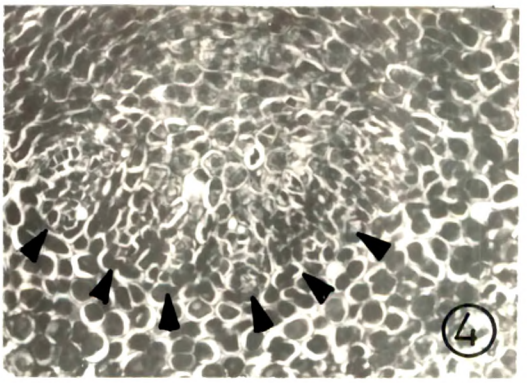
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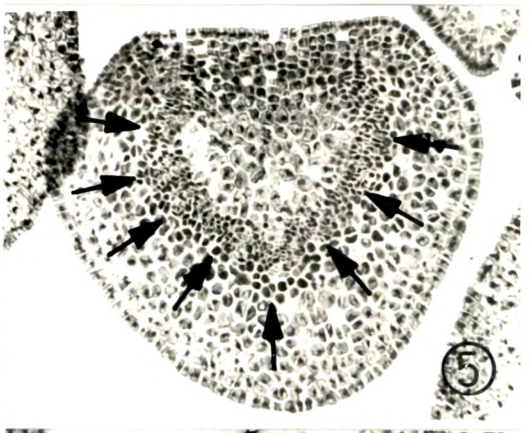
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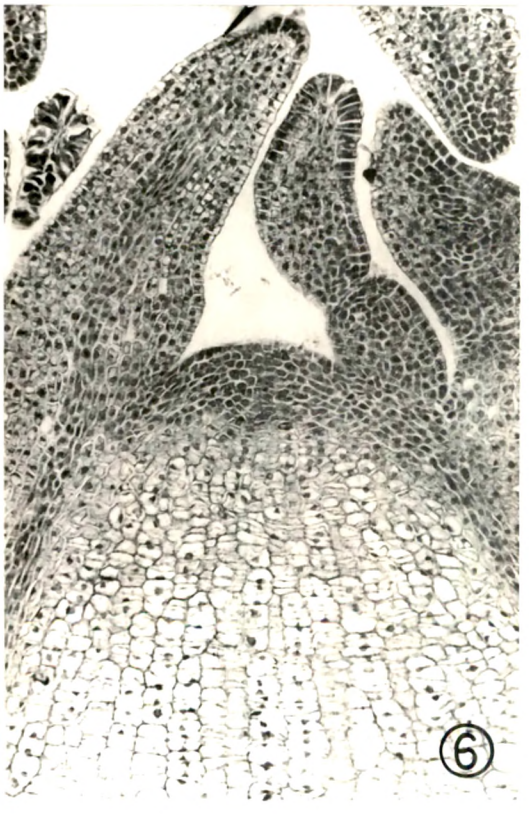
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Plate V

Fig. 7. Longitudinal section of the shoot apex showing the trace procambium (arrow) of a young leaf primordium. x 164.

Figs. 8-13. Transections of the petiole.

Fig. 8. Showing the vascular meristematic arc. x 142.

Figs. 9-13. A portion of vascular system enlarged.

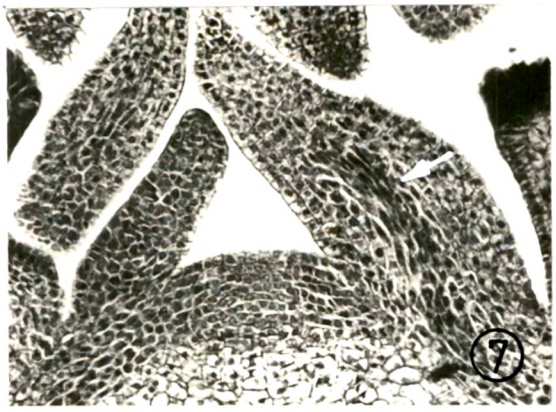
Fig. 9. Vascular strands show narrow procambial cells (asterisks). x 340.

Fig. 10. Radial seriation of the procambium (PC). x 200.

Fig. 11. Vascular strand shows procambium (PC) in between developing xylem and phloem. Periclinal divisions in the procambium are evident. x 380.

Fig. 12. Vascular strand during early stage of differentiation. Note the protoxylem element (PX) and protophloem sieve tube elements (PPh) arranged at the two poles separated by undifferentiated procambium. x 700.

Fig. 13. Early stage of metacambium (MC) differentiation. x 234.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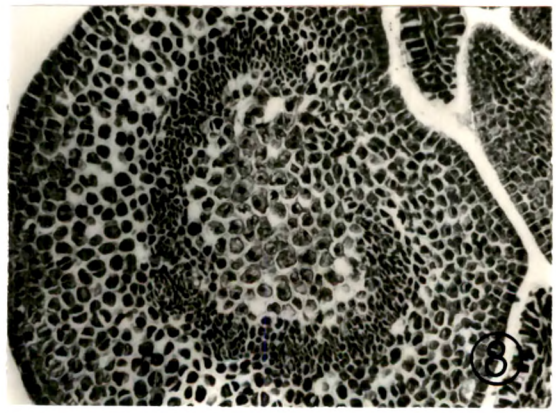
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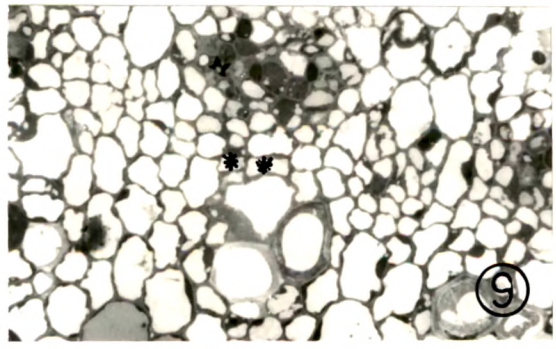
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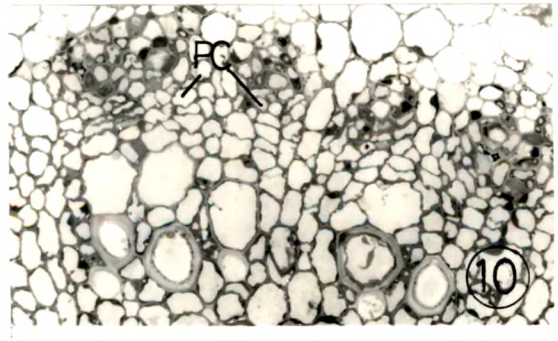
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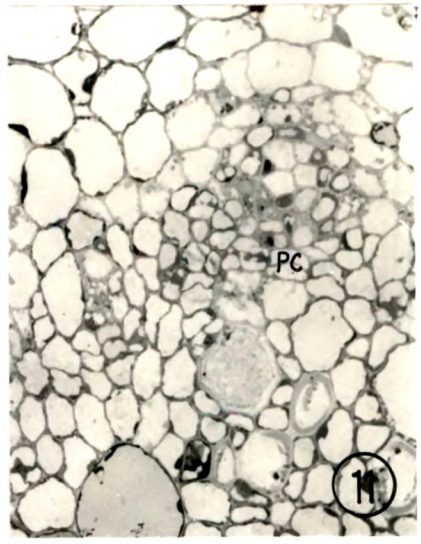
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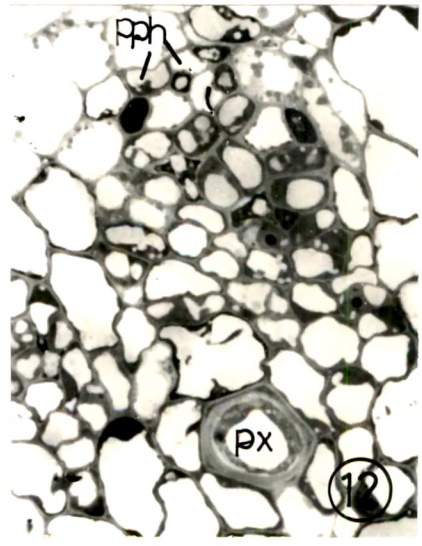
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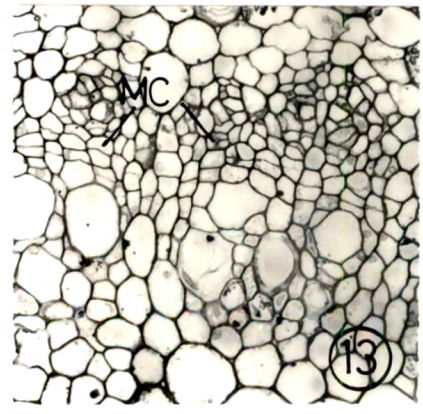
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Plate VI

Figs. 14-17. Transections of the petiole. A portion enlarged.

Fig. 14. Early developmental stage of the metacambium (MC). x 170.

Fig. 15. Vascular strand from a 5.6 cm long petiole showing 3-4 layered metacambium (MC).x 187.

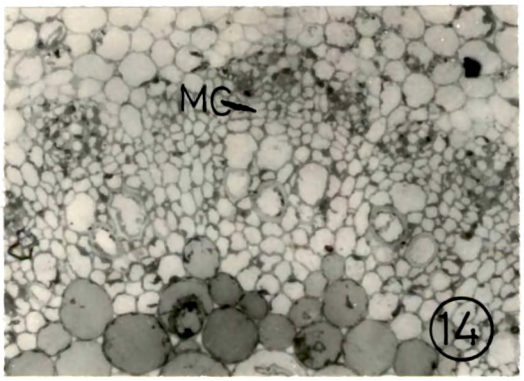
Fig. 16. Vascular strand from a 8.6 cm long petiole showing 3-5 layered metacambium (MC). x 180.

Fig. 17. Vascular strand shows interspersed parenchyma (arrows). x 86.

Figs. 18 and 19. Longitudinal sections of the metacambium.

Fig. 18. Shows the phragmoplasts (arrows) in a metacambial cell. x 400.

Fig. 19. Enlarged view. Note round nuclei with nucleolus. x 1000.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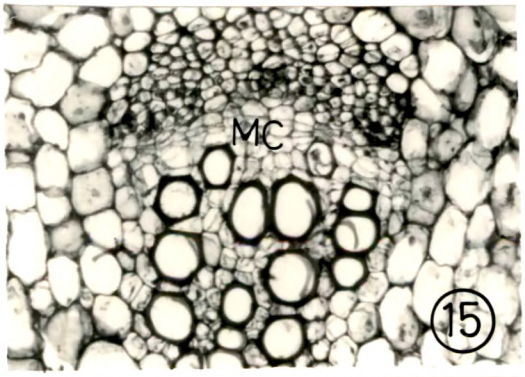
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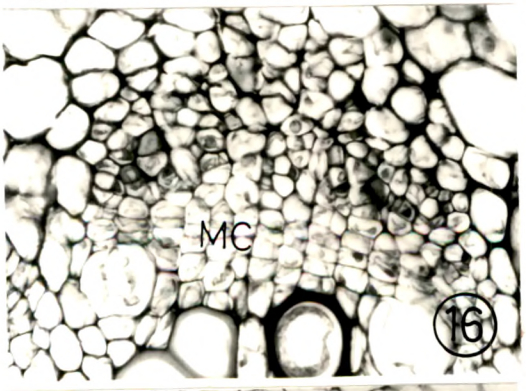
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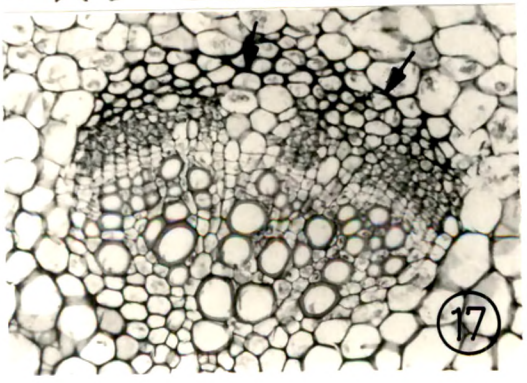
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Plate VII

Figs.20-24. Longitudinal sections of the procambium and metacambium in the petiole.

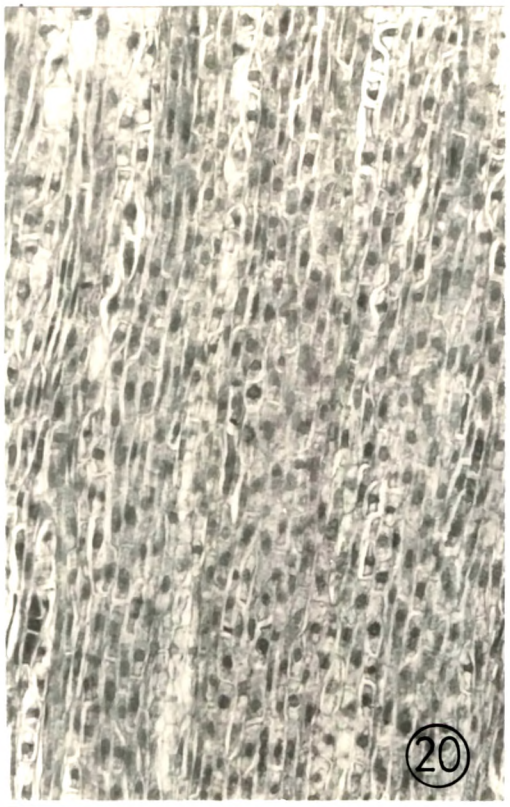
Fig. 20. Procambium in the early stage of development. x 320.

Fig. 21. Enlarged view of procambial cells. x 450.

Fig. 22. Radial longitudinal section showing the procambial cells (PC) in radial rows. Note the rounded end walls (arrow). x 650.

Fig. 23. Metacambium in a 5.6 cm long petiole. x 400.

Fig. 24. Metacambium in a 8.6 cm long petiole. x 238.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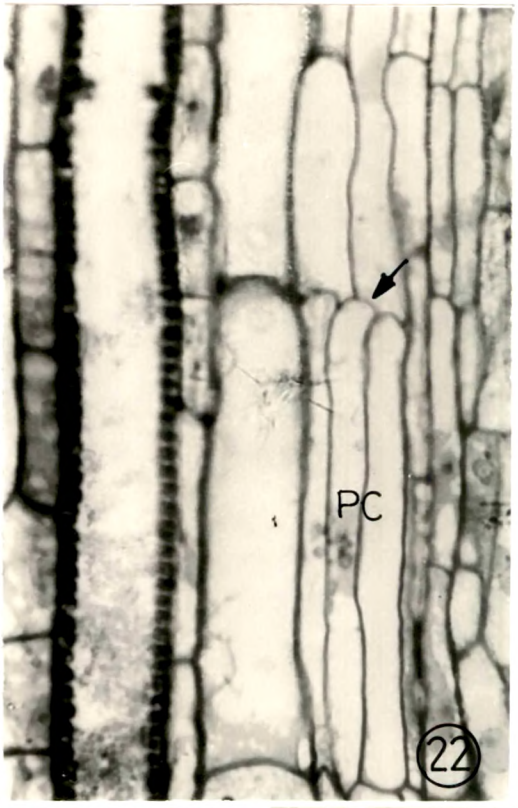
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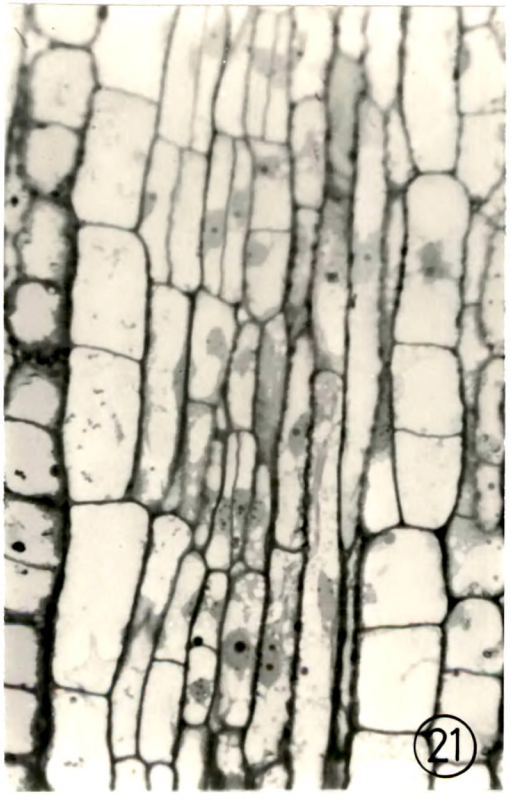
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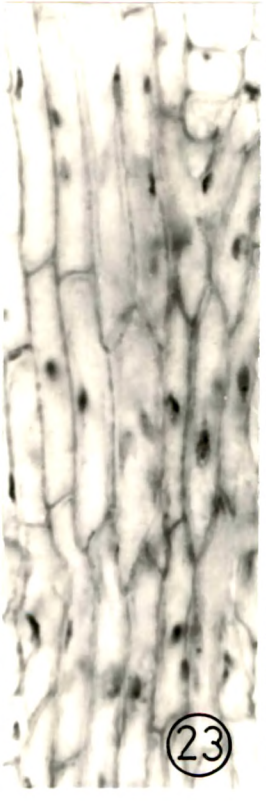
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Plate VIII

Figs. 25-31. Transections of the petiole during its different developmental stages.

Fig. 25. Vascular strands in the early stage of differentiation. Note protoxylem (PX) and protophloem (PPh) at the two poles. x 200.

Fig. 26. Vascular strand showing periclinal divisions in the procambium (arrow). x 275.

Fig. 27. Vascular strand shows differentiating metaxylem elements (MX). Periclinal divisions are not clearly evident in the procambial cells. x 515.

Fig. 28. Radial seriation resulted from successive periclinal divisions in the procambium (asterisks) during early stage of metaxylem differentiation. x 575.

Fig. 29. Vascular strand showing the protoxylem and metaxylem elements in a row. Arrow points to the stretched protoxylem element. x 98.

Fig. 30. Vascular strand during the primary - secondary transition stage. Note the lignification of xylem parenchyma cells (arrow). x 162.5.

Fig. 31. Liginification of xylemm parenchyma and interfascicular parenchyma. Note the thickened protophloem fibres on the outer periphery of the strand. x 80.

Figs. 32 and 33. Longitudinal sections of the petiole during the transition.

Fig. 32. Two young tracheary elements in a row presumably differentiated by a transverse division (arrow) in the metacambial cell.

Fig. 33. A metacambial cell directly transforms into a vessel element (arrow). Note the two fusiform ends. x 136.

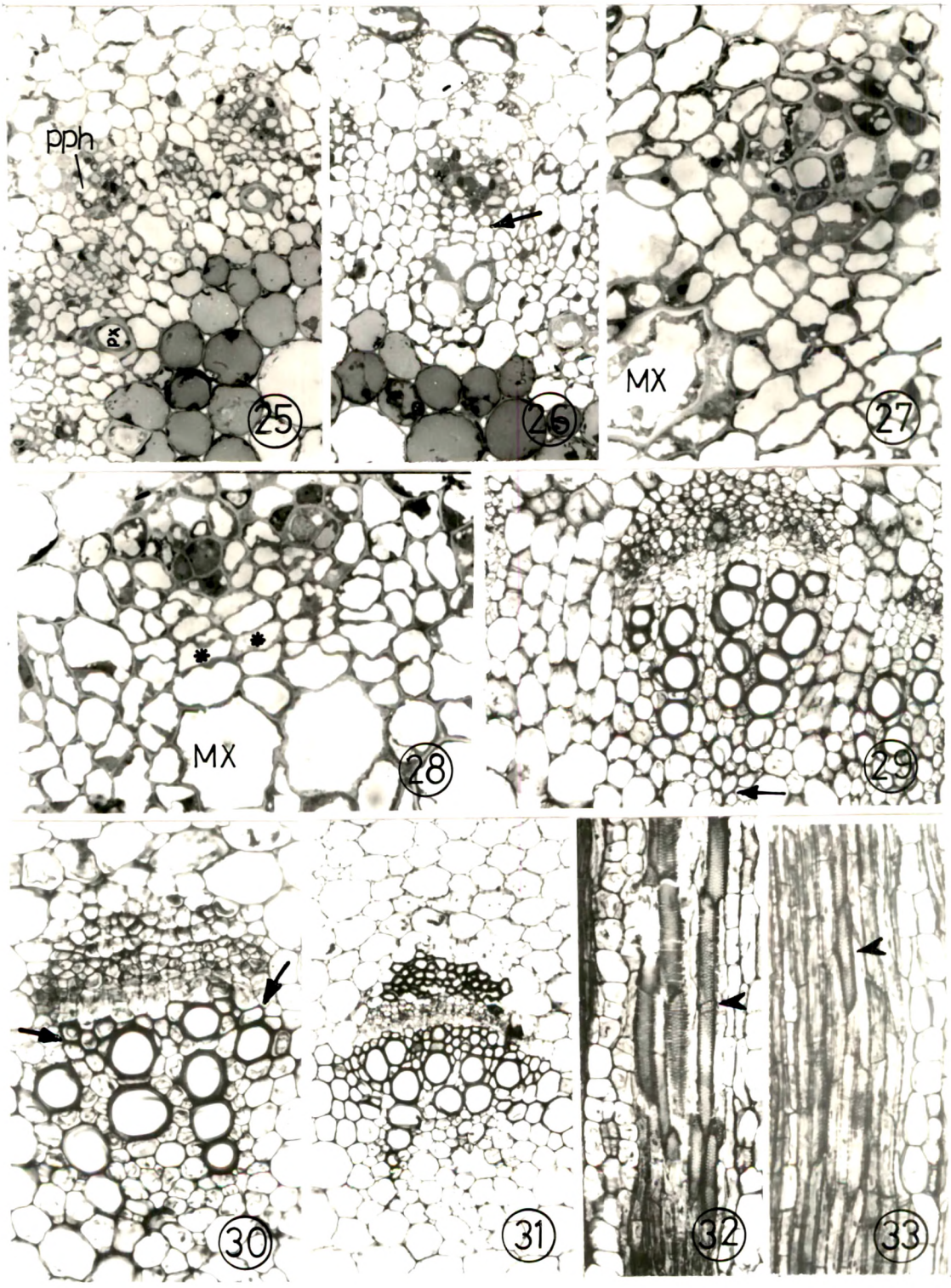
pph
ax
25
26
MX
27
MX
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Plate IX

Figs. 34-36,39. Transections of petiole.

Figs. 34 and 35. Polarized light micrographs.

Fig. 34. Young petiole showing birefringent meta-xylem elements (arrows). Although phloem bundle cap fibres are differentiating their walls do not exhibit birefringence. x 110.

Fig. 35. Mature petiole showing secondary xylem consisting of thickened xylem parenchyma, interfascicular parenchyma and vessels. Note the well developed lignified phloem cap fibres. x 69.

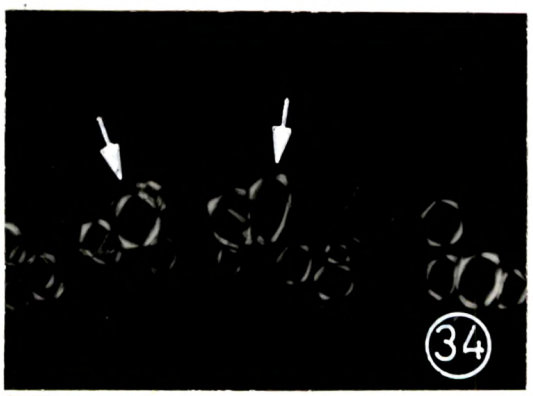
Fig. 36. Portion of a vascular strand from a mature petiole showing 3-4 layered vascular cambium (arrows point to radial walls). x 900.

Figs. 37 and 38. Longitudinal sections of the vascular cambium in the petiole.

Fig. 37.. Non-storied cambium consisting of only fusiform initials (FI). x 125.

Fig. 38. Enlarged view of a portion of the fusiform initial showing the beaded wall. x 600.

Fig. 39. Senescent petiole showing thick radial walls of the cambial cells (arrow). x 3900.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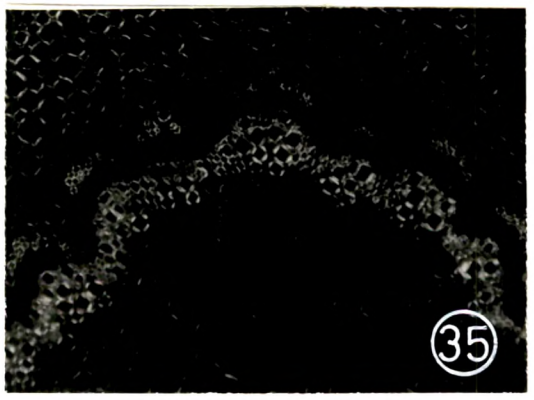
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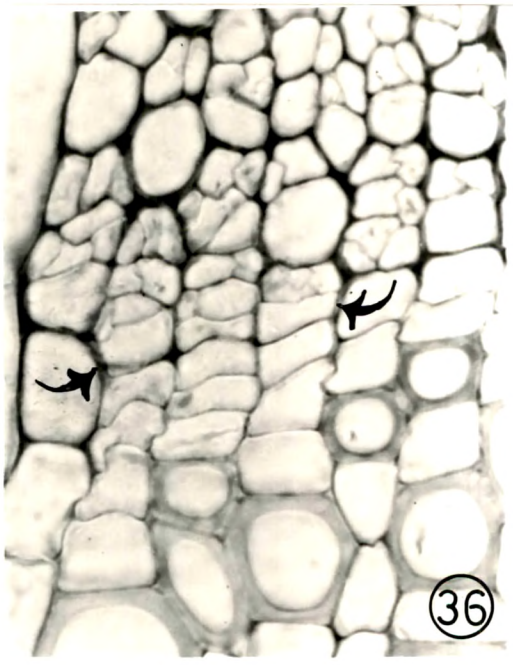
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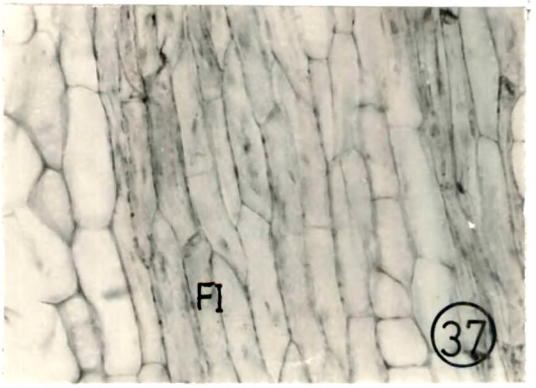
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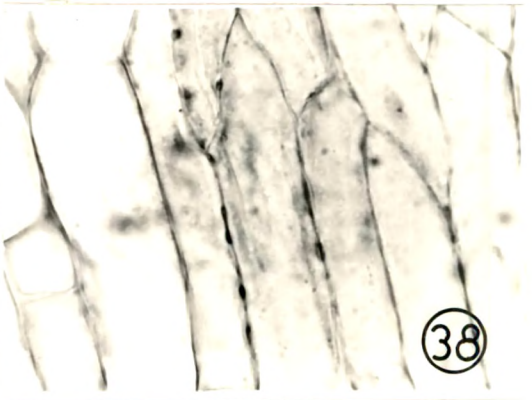
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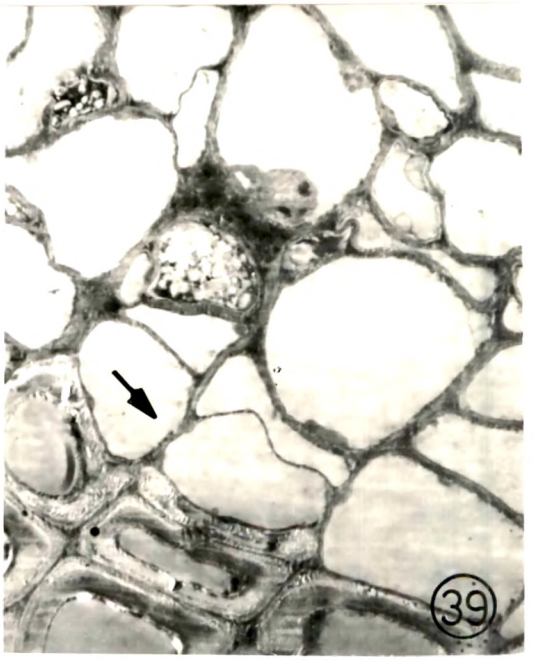
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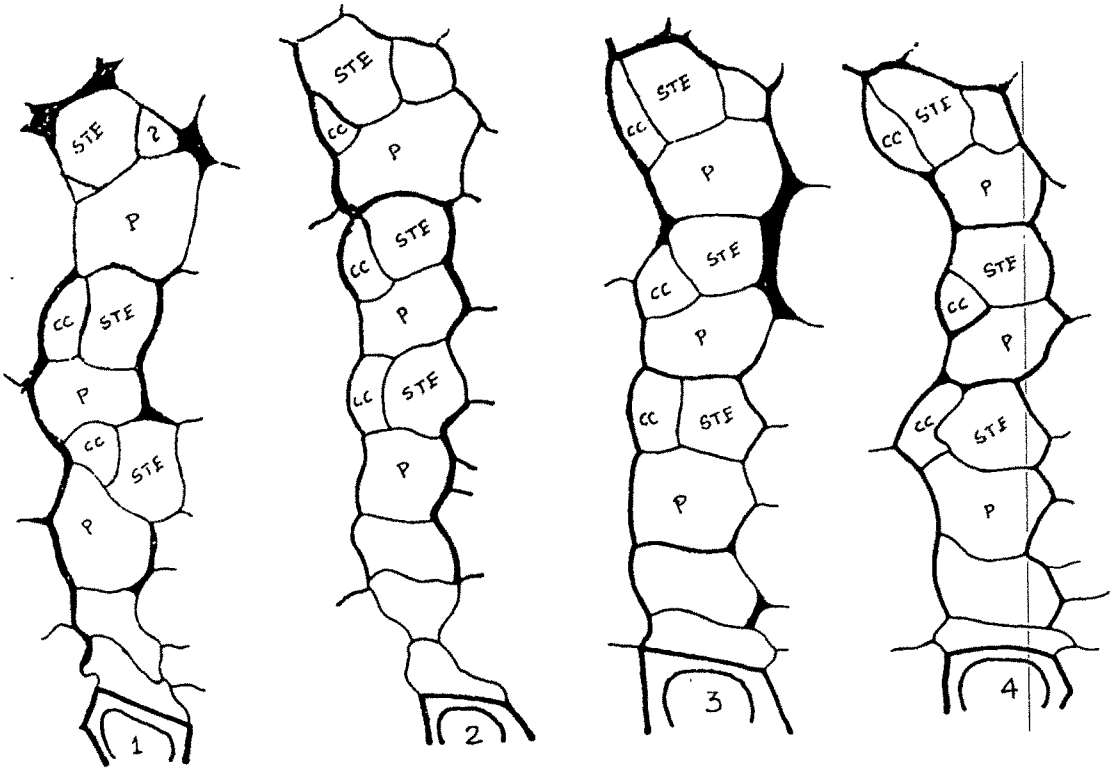
Figure 40. Analysis of radial file of phloic cambial derivatives. Arabic numerals 1 to 7 represent a phloem complex at an interval of 10 microns (Bar = 10 μm).
(STE = Sieve tube element; CC = Companion cell; P = Phloem parenchyma).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P
1
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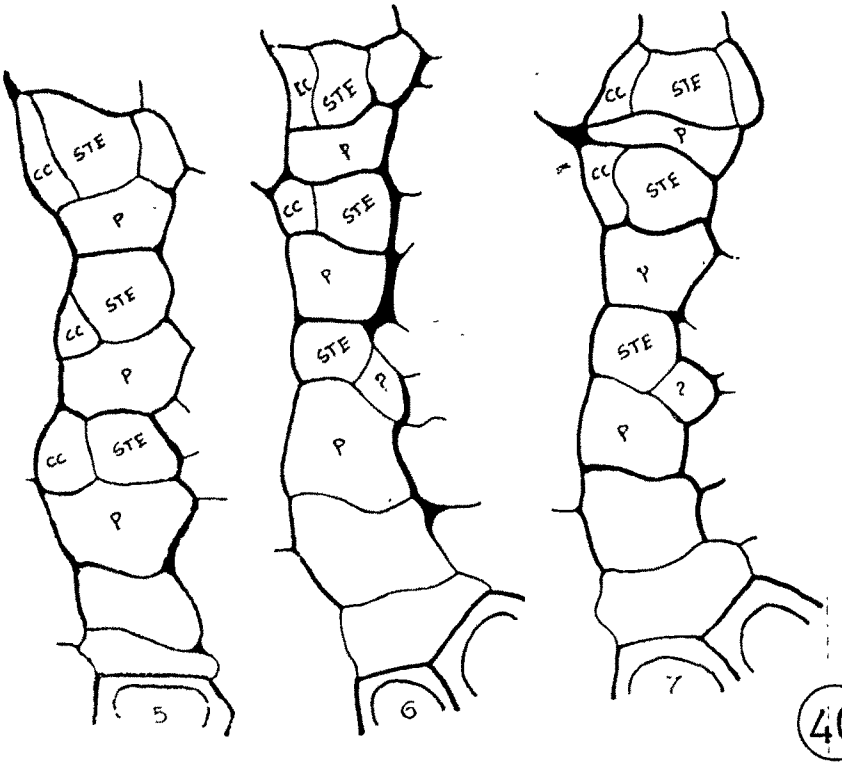
STE
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Plate X

Fig. 41. Transection of the bark, a portion enlarged to show the fusiform initials (FI) and rays (R). x 232.

Figs.42-45. Longitudinal sections of the bark showing vascular cambium (42,43) and petiole showing wound cambium (44,45).

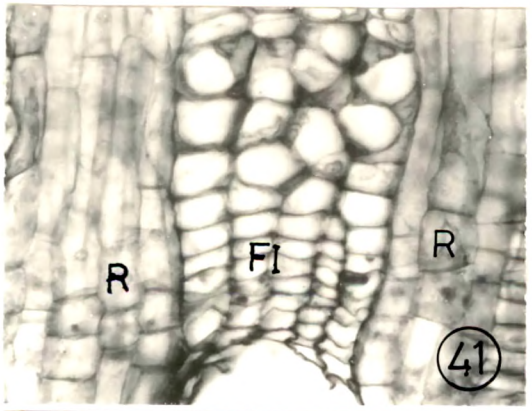
Fig. 42. Fusiform initials (FI) and rays (R). x 196.

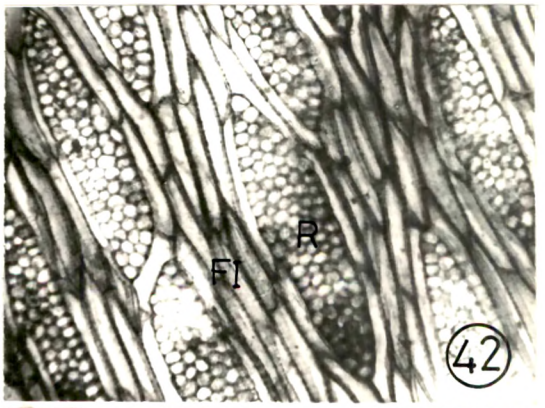
Fig. 43. Enlarged view of fusiform initials and ray (R) cells. Note the beaded radial walls. x 242.

Fig. 44. Showing differentiation of tracheary elements from fusiform initials (arrows). x 156.

Fig. 45. Fusiform initials and rays (arrows). x 66.

Fig. 46. Transection of a wounded petiole showing wound callus (arrow). x 17.


R
Fl
R
41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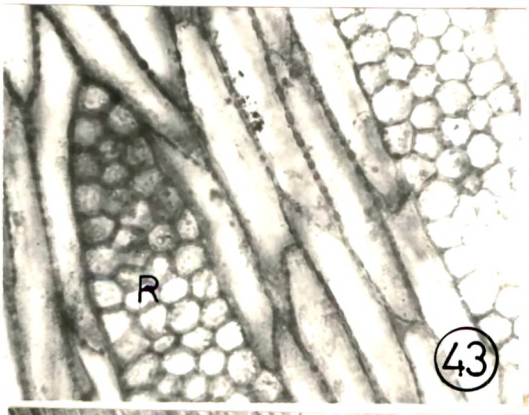
R
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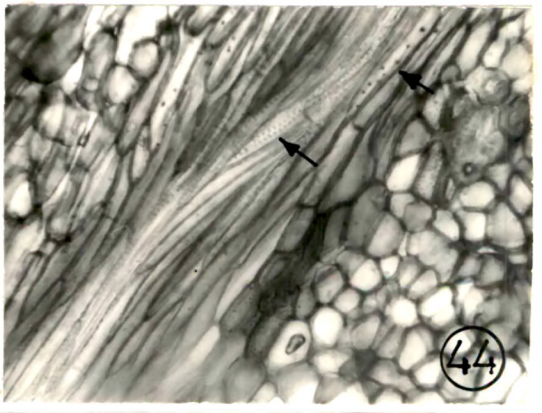
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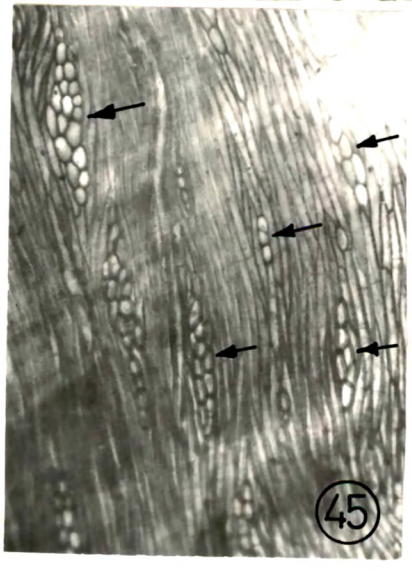
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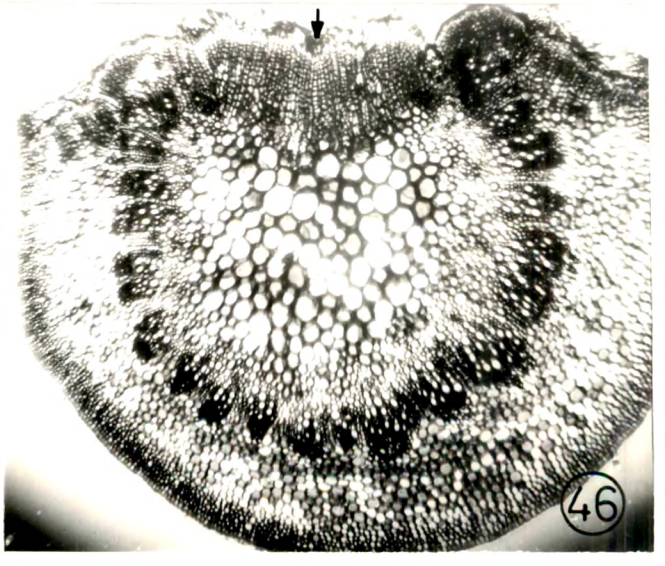
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SECTION III

3.1.10 Development of the phloem in the petiole

The establishment of procambium in a developing leaf is followed by the differentiation of some of its cells into primary phloem and primary xylem. It is mentioned earlier that the procambium in the petiole of Crataeva differentiates acropetally. Here, the first phloem elements also differentiate acropetally along the outer periphery of the procambial trace strand. The first sieve elements are noticed in the petiole at the third node from the shoot apex. They are initiated towards the cortical side of the strand. In a given vascular strand in the petiole, protophloem differentiation begins with one or two sieve tube elements followed by the development of the protoxylem tracheary element (fig.25, Section II). New sieve tube elements differentiate in a centripetal direction. The earliest sieve elements are comparatively smaller than other successively formed ones. They are readily discernible because of their comparatively thick walls and clear, empty lumen (fig.1). The clear wide lumen contrasts strikingly with the rather densely stained protoplasts of the adjacent cells. The companion cell for each sieve tube element may be present or absent. The sieve plate is simple.

3.1.10.1 Structure of the phloem in a 1 cm long petiole

Young vascular strands which traverse more or less without much anastomosis in the petiole were selected at random for the study of phloem differentiation and structure. Since all the strands in a given petiole do not develop simultaneously the state of procambial differentiation is variable in them, but every strand progresses through the same ontogenetic sequence of differentiation. My observations are mainly confined to the 2-4 strands at the abaxial region of the petiole and these observations have been compared with those of the other vascular strands.

After the differentiation of few sieve tube elements the radial seriation is evident in the procambium (fig.26, Section II). Sieve tube elements appear solitary or in pairs, rather randomly distributed amongst the parenchyma cells, a few of them have short functional existence. They mature during early elongation of the petiole and, become stretched and later obliterated.

The cell wall of the mature protophloem sieve elements appears denser than that of the phloem parenchyma cell. The sieve plate is simple and transverse to oblique. The P-protein with other contents accumulate

at the sieve plate in the form of a plug formed on one side of the sieve plate (figs. 2 and 3). Sieve areas on lateral walls are aggregate, scattered. The sieve tube element is with or without a companion cell. When present, the latter is as long as its associated sieve element.The companion cell is uninucleate with dense cytoplasmic contents. The nucleus is oval, with a nucleolus. The common wall between a companion cell and a sieve tube element shows sieve areas on the sieve tube element wall. The companion cell lacks starch grains.

3.1.10.2 Phloem in a 5.6 cm long petiole

As noted earlier, metacambium is observed in a 5.6 cm long petiole. Once the metacambium within a strand has attained a certain level of distinct morphological identity, then some of its derivatives differentiate to metaxylem and metaphloem. The metaxylem shows radial pattern of arrangement whereas the metaphloem does not show any specific pattern of orientation. Due to the surging artefact the contents of the mature metaphloem sieve tube elements appear contracted and generally aggregate towards the sieve plate in varying densities. The linear, scattered sieve areas ranging from 2-11 occur on lateral walls.

In most histological preparations the contents of the mature sieve tube element aggregate at their ends as slime plugs. Their formation arises from the surging artefact gives an indication that the sieve elements in question were active. The pattern of plug formation may reflect the status of content distribution in a normal mature sieve tube element. A parietal distribution of P-protein in mature sieve tube members has now been reported for a number of plants (Evert, 1990). Such elements are expected to be least affected by the surging phenomenon that occurs when sieve tubes are severed, reflecting the normal distribution of P-protein within the lumen. It is presumed that the concave side in figures 4-6 is due to the parietal position of P-protein in mature sieve tube elements and the string-like extensions on both sides may be the remnants of parietal cytoplasm. The mercuric bromophenol blue and coomassie brilliant blue stain the slime plugs indicating a positive reaction for phloem specific proteins (fig.7). Plastids, mostly in the form of starch also accumulate at the sieve plate but are occasionally distributed in the entire lumen.

The metaphloem sieve element is generally associated with one or two companion cells. Longitudinally a companion cell usually extends from one end of a sieve tube element, sometimes when a vertical row of two or

rarely more companion cells are present, the length of one companion cell is almost half of the length of the sieve tube element (fig.8). In contrast to the sieve tube element, the companion cell retains its nucleus at maturity. The stains used for phloem-specific proteins show a uniform staining for slime and companion cell nucleus and cytoplasm (figs. 9 and 10). The deeper staining of the companion cell nucleus and cytoplasm may probably be due to a substance similar to the phloem-specific proteins. Starch grains are absent in the companion cell.

Structure of the phloem in a 8.6 cm and 11 cm long petiole closely resembles with that described earlier. Hence, its description is omitted.

3.1.10.3 Structure of the phloem in a 14 cm long petiole

Earlier, a mature petiole is defined as the one which ceased to elongate and here the 14 cm petiole is considered mature because it did not show further elongation.

The characters accompanying the transition of procambium to cambium in the middle region of the petiole are lignification of xylem and interfascicular parenchyma and protophloem fibres and the differentiation of few

xylem fibres. But in the pulvinal base and distal region the identification is exclusively based on the fusiform nature of the cambial cells and development of short tracheary elements almost equal to the length of fusiform initials.

Although a few early secondary elements may be observed differentiating during the final stages of petiole elongation, they do not mature until petiole elongation has ceased. At this time these elements attain their final length and undergo lignification. Lignified elements can be readily detected by their birefringent wall in the polarized light (fig.35, Section II). Interfascicular parenchyma also shows thickening and lignification during transition progressing centripetally. Although these events can be correlated with the cessation of petiole elongation, the differentiation occurs gradually within the maturing petiole, the events do not necessarily coincide with one another.

During the elongation of the petiole the parenchymatous precursors of phloem fibres outlying the periphery of the vascular strands in the middle region enlarge (figs.11-14). These cells gradually develop secondary wall during the last phase of elongation and develop as fibres in the mature petiole (figs.15-19). In contrast to the middle region of the petiole, the distal

and proximal pulvinal region in the same petiole does not show fully developed protophloem fibres. Neither lignified interfascicular parenchyma nor birefringent xylem fibres are present in these regions.

In a mature petiole fascicular cambium produce radially arranged complexes of secondary phloem in the middle region (fig.36, Section II). Radial arrangement is less evident in the pulvinal and distal regions. Derivative of a fusiform initial divides and arranges to form a component of the complex of phloem cells which include sieve element, companion cell and phloem parenchyma. Each phloem complex has 5-8 tiers of conducting elements and adjacent complexes are distinguished by thick radial walls.

The secondary phloem sieve tube elements vary in length. Ontogenetically they may be shortened by the occasional transverse divisions in the fusiform initials. A sieve tube element usually has one companion cell which is as long as its element (fig.20); rarely two companion cells are noticed. In certain vascular strands a row of radially elongated interspersed parenchyma cells extend from the cambium upto the primary phloem region (fig.21). Where interspersed parenchyma cells are present, companion cells are noticed contiguous to them. The

common wall between a sieve tube element and a companion cell appears curved (fig.22). The sieve plate is mostly at the oblique end walls.

3.1.10.4 Obliteration of sieve elements

In a vascular strand vascular differentiation begins with the development of a few protophloem sieve elements followed by protoxylem elements. New sieve elements differentiate centripetally. After the formation of one or two sieve elements, further differentiation of more protophloem sieve elements takes place. At this stage first signs of obliteration are observed (fig.12). Protophloem differentiation continues till the radial seriation in the metacambium becomes evident. These sieve elements also have a very short functional existence, they mature during early elongation of the petiole and, once the petiole reaches about 5.6 cm in length, become stretched and crushed. This portion of the phloem is considered protophloem. Protophloem sieve elements are prone to stretching and elongation because of their occurrence in the elongating petiole.

After the obliteration of protophloem sieve elements, the parenchyma cells surrounding the sieve elements enlarge and group with the parenchymatous

precursors of phloem fibres (figs.12-15). When the sieve tube elements are completely crushed between the enlarging parenchyma cells, thickened places with callose spots temporarily indicate the former position of obliterated sieve elements (fig.14).

After the first thirteen to fifteen days of growth the petiole ceased elongation during which the primary phloem development was completed, afterwards the secondary phloem development began. During the elongation of the petiole the protophloem sieve elements obliterate and protophloem is mostly represented by protophloem fibres and few parenchyma cells. The metaphloem remains relatively unchanged till about one month. Then the crushing of sieve tube elements in the metaphloem becomes evident - first in the early metaphloem elements and later centripetally in the late formed ones. Secondary growth becomes well established in the mature petiole by about two months. By the end of the growth period of the leaf i.e. after 4-5 months, metaphloem becomes completely functionless.

The process of obliteration is similar in protophloem and metaphloem sieve elements. Obliterated sieve elements are stretched with a narrow lumen and heavy deposition of callose at the sieve plate (fig.24).

Obliterating sieve elements have empty lumen with moderate deposition of callose (figs. 25, 26). When the petiole becomes senescent all the sieve elements are found to be blocked by callose. The cell wall of the sieve elements become comparatively thick (fig.23) and crystals may develop in the interfascicular parenchyma in the phloem region, before the leaf falls occurs.

3.1.10.5 Primary phloem and secondary phloem : a comparison

The procambial cells in the petiole have mostly transverse end walls and the primary sieve tube elements are characterized by transverse or nearly transverse terminal walls (fig.27). The secondary sieve tube elements, derived from fusiform initials that may divide by nearly transverse walls, show mostly oblique to nearly transverse terminal walls (fig.28).

The primary sieve elements elongate during their ontogeny, becomes longer than the initials from which they are derived. The secondary sieve elements do not elongate, and, because of occasional transverse divisions in their mother cells may become shorter than the initials.

The primary sieve tube elements like other members of the longitudinal system of the primary phloem are narrower than the corresponding elements of the secondary phloem. Dimensional relationship of the protophloem, metaphloem and secondary phloem in the petiole is given in Table III.

3.1.10.6 Secondary phloem production in the mature petioles

Measurements indicate that during the post elongation period the mature petiole produces secondary phloem and secondary xylem (see fig.45). The cambial region is 2-3 cell layers thick, regardless of the months and position within the petiole. The peak of secondary phloem production is found to be in the second month and then the activity gradually declines. Considering the time intervals involved i.e. 4 months, there is no evidence for a dramatic burst of phloem production in mature petioles. Instead, there is a slow, rather gradual production of phloem throughout the growing season. In Crataeva with advancing leaf age the radial width of phloem cells remains fairly unchanged despite monthly production of new phloem cells along the entire length of the vascular strands.

3.1.11 Secondary phloem in the bark of Crataeva

The secondary phloem of Crataeva in the stem consists of sieve tube members, companion cells and phloem and ray parenchyma cells (fig.29). Sclerenchyma cells are generally absent in the conducting phloem. The conducting phloem is that part of the phloem containing mature sieve tube members, presumably involved in active conduction of assimilates (fig.30). As seen in transection of the functional phloem, the phloem cells of the axial system occur in orderly, radial series. The sieve elements which are usually the large cells, have a clear appearance, except near their ends or at the sieve plates, where slime and plastids are commonly accumulated. Companion cells which are small occur at the corners of sieve elements and commonly have relatively dense protoplasts. Phloem parenchyma cells, which are scattered more or less uniformly throughout the phloem increment, are usually intermediate in size between the sieve elements and companion cells. The rays are multiseriate. Sieve elements are commonly associated with two or more companion cells (fig.31). End walls of the sieve elements are much inclined and bear simple sieve plates. Visible contents of the pores vary in appearance with the distribution and appearance of slime within the elements.

The distribution and appearance of slime in the mature sieve elements examined during this study vary and range from amorphous slime plugs at the sieve plates in some elements to slime bodies in others. These slime bodies are proteinaceous as indicated by mercuric bromophenol blue staining and appear to be a P-protein body as defined at the ultrastructural level (Cronshaw and Sabnis,1990). Slime bodies are round to oval in shape (fig.32). Senescent nuclei are found in most of the mature sieve elements with fully developed sieve plate. Some of these nuclei are quite swollen and lightly stained (fig.33).

Lateral sieve areas occur on longitudinal walls of the sieve elements. Old sieve elements show the presence of definitive callose. With the cessation of function of the sieve elements and their concomitant loss of turgor, the contiguous parenchyma cells gradually increase in size and appear rounded in transections. Some parenchyma cells become sclerified in the non-conducting phloem. Only outer bark contain sclereids. Rays become dialated in the non-conducting phloem.

A comparison of the secondary phloem elements in the petiole and bark is shown in Table IV.

3.1.12 Wound sieve elements

When the petiole is cut in such a way as to sever the vascular strands, it was possible to observe the reestablishment of vascular connections during a subsequent recovery period (fig.34). Experimental interruption of the sieve element continuum indicates the development of laterally deviating wound sieve tubes within 10-12 days in a definitive area in the petiole. The timing of wound phloem development depends on the intensity of strand severence. Wound sieve elements are products of parenchyma cells, which becomes meristematic on wounding (fig.35). Wound sieve tubes are initiated mostly above the wound. It is observed that wound sieve elements differentiate from the wound cambium (fig.36). They are continuous with the sieve elements situated near the cambium above and below the cut.

The differentiation of the wound sieve elements proceeds downwards from the region above the cut. Its shape is mostly determined by the outline of the original cell and the angle by which this cell is subdivided during the initial meristematic divisions. The course of wound sieve tubes which bypass the wound is seldom straight. Due to the predominantly parallel orientation of the second and later divisions, wound sieve elements form often an arc-like or otherwise a bent file of small cells (fig.38). Wound sieve element is mostly associated with a companion cell (fig.37).

3.1.13 Comparison between wound sieve elements and secondary sieve elements in the petiole

Apart from their origin, the shape and size of the mature wound sieve element are comparable to those of the secondary phloem sieve element in the petiole (fig.39). Starting with the unequal division of a cell that gives rise to a sieve element and its companion cell, these events include the loss of nucleus at maturity, development of sieve pores, sieve areas etc. These characteristics and the association of a companion cell, appear to be essential for cells in order to be able to translocate assimilates.

3.1.14 MTT localization of metabolic activity in the phloem

The ability of metabolic reduction, which is assumed to reflect the rate of physiological activity, was investigated by the use of thiazolyl blue (MTT). Cells which produce sufficient mitochondrial NAD(P)H are able to reduce the dye, producing dark blue colouration.

In contrast to the pith and cortex, the xylem parenchyma cells around the vessels stained darkly (fig.40). Of the sieve tube/companion cell complexes particularly the companion cells stained strongly

(figs.41 and 43). The companion cells which possess numerous mitochondria stained dark blue. The mature sieve tube elements having scanty cytoplasm and less mitochondria stained lightly. Developing sieve elements are rich in cytoplasm and organelles, stained more or less uniformly. Obliterating sieve elements, in turn did not show any indication of metabolic activity (fig.41).

The fibrous elements showed only weak staining with thiazobyl blue or absence of staining. However, young fibres did show intensive staining indicating high metabolism (fig.42). Vein cells stained heavily compared to mesophyll cells (fig.43). Mostly staining of mesophyll cells was absent. In the veins companion cells were most heavily stained as shown in the petiole.

Table III : Dimensions of the protophloem, metaphloem and secondary sieve tube elements and companion cells during the elongation of the petiole. Observations are confined to the middle region. Dimensions are in μm. Value in brackets shows the range.

Length of the petiole in cm	Length						Width					
	Protophloem		Metaphloem		Secondary phloem		Protophloem		Metaphloem		Secondary Phloem	
	STE	CC	STE	CC	STE	CC	STE	CC	STE	CC	STE	CC
0.3	62.8 (47.10-87.92)	-					3.58 (3.12-4.71)					
1.0	86.35 (56.52-103.62)	84.78 (54.95-104.48)					3.61 (3.14-4-71)	2.20 (1.57-3.14)				
5.6			119.32 (62.80-151.30)	96.32 (40.28-151.30)					8.32 (7.85-9.42)	3.24 (3.14-4.71)		
8.6			86.12 (40.82-142.58)	96.05 (48.67-142.58)					7.79 (6.28-9.42)	3.64 (3.14-4.71)		
11.3			91.02 (57.81-149.15)	88.34 (47.1-125.60)					7.15 (6.28-9.42)	3.53 (3.14-4.71)		
14.0					125.60 (47.1-139.73)	119.32 (47.1-135.02)					9.08 (7.85-12.32)	4.08 (3.14-4.71)

Table IV : A comparison of the secondary phloem elements in the petiole and bark (both July collections). Dimensions are in μm

	Petiole	Stem
Tissue types	Axial system	Axial system and ray system
Components of the secondary phloem	Sieve tube elements companion cells and axial phloem parenchyma.	Sieve tube elements, companion cells, Axial parenchyma, Ray parenchyma and sclereids.
Sieve tube element		
Length	72.0	173.64
Breadth	5.8	25.73
End wall	Inclined	Inclined
Sieve plate	Simple	Simple
Diameter of the sieve plate	6.1	28.73
Nucleus	Mature element with scanty contents	Nucleus observed in mature sieve elements.
Slime body	Not common	Common
Companion cell		
Number	Mostly one or two	Two or more, rarely one
Length	68.2	70.97
Breadth	3.40	6.53

Plate XI

Fig. 1. Transection of the young petiole. A portion enlarged to show the differentiation of first sieve elements in the procambial strand (arrow). x 693.

Figs. 2-10. Longitudinal sections of the sieve elements.

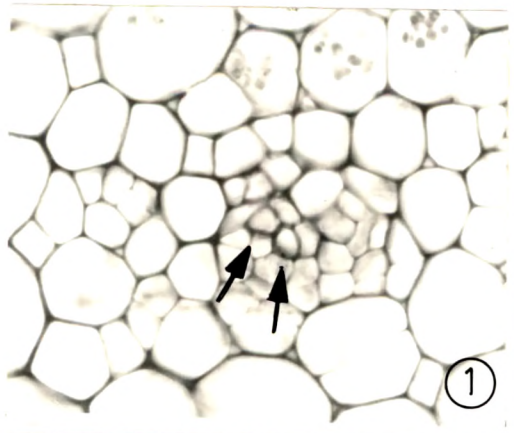
Figs. 2 and 3. Protophloem sieve elements. Note the transverse end wall (at arrow) of the sieve elements. Fig.2. x 333; Fig.3. x 500.

Figs. 4-6. A portion of the sieve tube element showing a plug with accumulation of P-protein and other contents. Note the concave inner side (arrow) and string like extensions on both sides. Fig.4. x 1000; Fig.5. x 1250; Fig.6. x 1000.

Fig. 7. Accumulation of P-protein alongwith starch grains (arrow) and other contents. x 1125.

Fig. 8. A metaphloem sieve tube element with two companion cells in a row. Arrow points to the nucleus. x 346.

Figs. 9 and 10. MBB staining to localize P-protein in the sieve elements (arrow). Note the densely stained nucleus and cytoplasm of the companion cells (arrow). x 600.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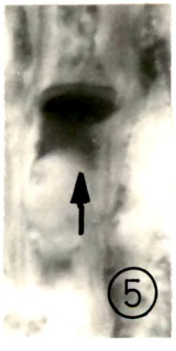
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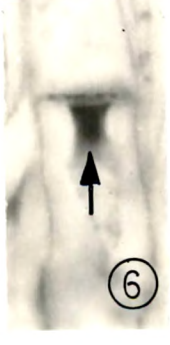
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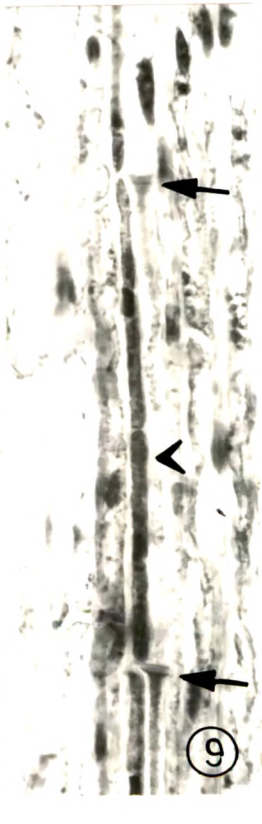
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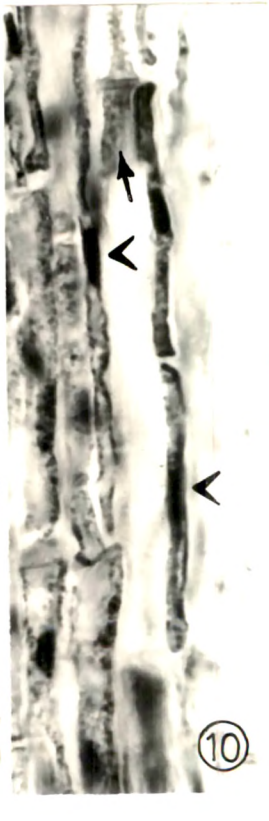
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Plate XII

Figs. 11-18. Transverse sections of the petiole. A portion enlarged to show the sequential development of protophloem, metaphloem and secondary phloem and protophloem fibres.

Fig. 11. Early stage of vascular strand development x 133.

Fig. 12. 5.6 cm long petiole. Parenchyma precursor cells of protophloem fibres grouped at the periphery. Arrows point to the obliterated protophloem sieve elements. x 250.

Fig. 13. 8.6 cm long petiole. Mostly only metaphloem is observed (MP = metaphloem). x 437.

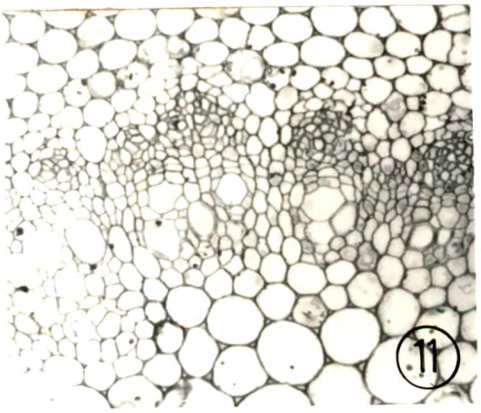
Fig. 14. 11.3 cm long petiole. Note the accumulated callose (arrow) in the obliterated sieve elements. x 287.

Fig. 15. Petiole before cessation of elongation. x 312.

Fig. 16. Petiole few days after cessation of elongation. Lignification of the fibres (F) is evident. x 240.

Figs. 17 and 18. One month old and four month old petiole respectively. Fully developed fibres are seen (SP = Secondary phloem). x 600.

Fig. 19. Longitudinal view of a pair of mature fibres. x 320.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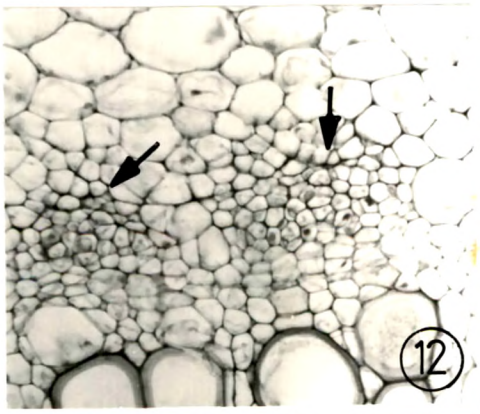
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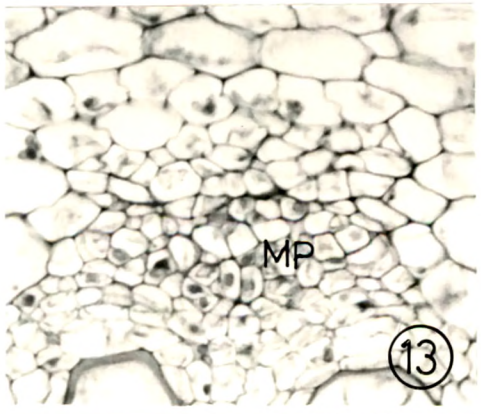
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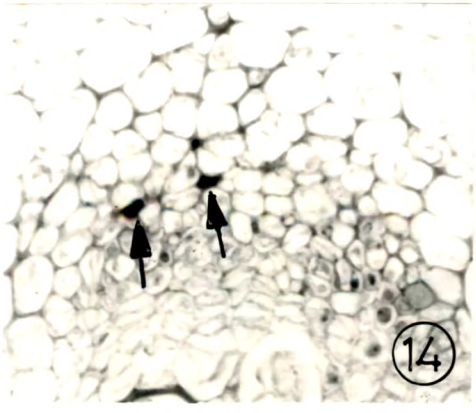
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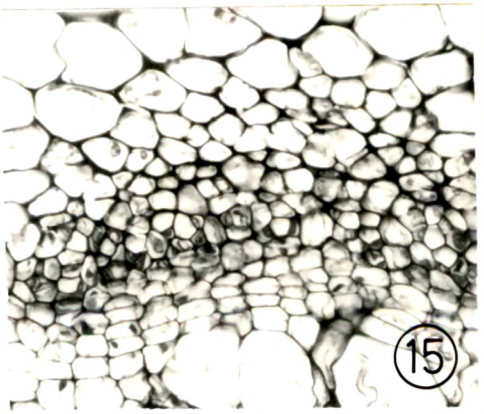
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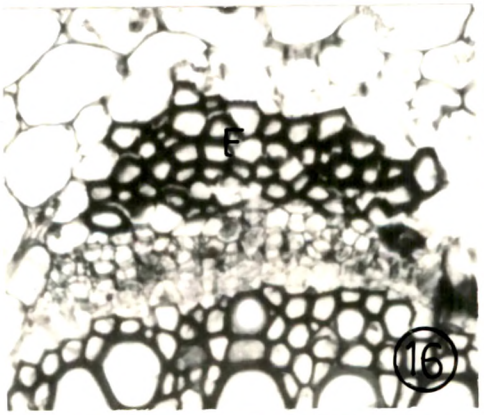
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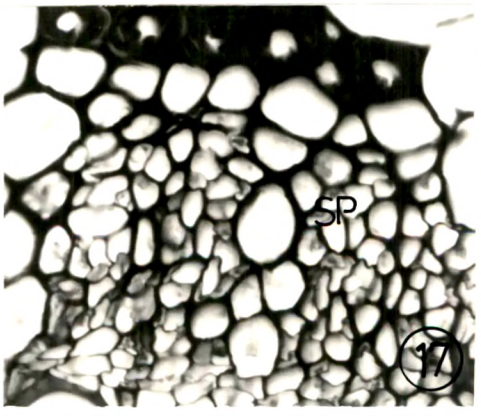
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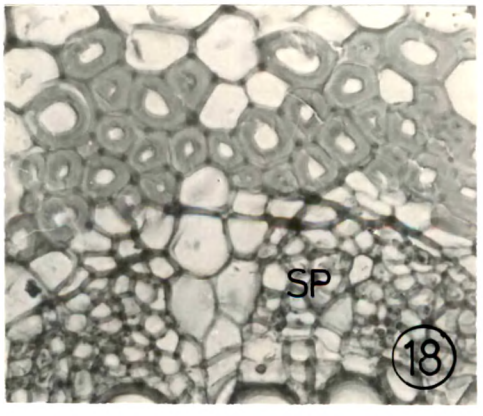
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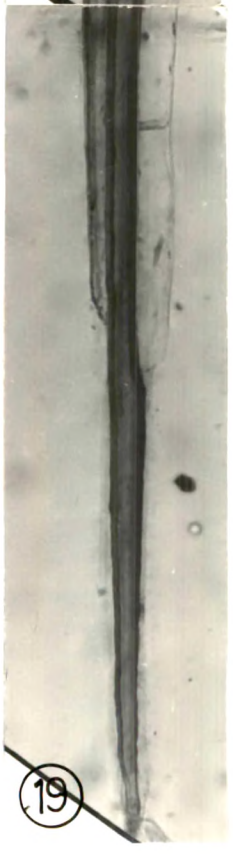
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Plate XIII

Fig. 20. A secondary phloem sieve tube element with a companion cell. x 600.

Fig. 21. A portion of the vascular strand from a mature petiole enlarged to show the interspersed parenchyma (IP) and contiguous companion cells (arrow head). x 240.

Fig. 22. Transverse section of a mature secondary sieve tube element. Note common wall between sieve tube element and companion cell (CC) is curved. x 24640.

Fig. 23. A senescent secondary sieve tube element with a companion cell (CC). x 45,000.

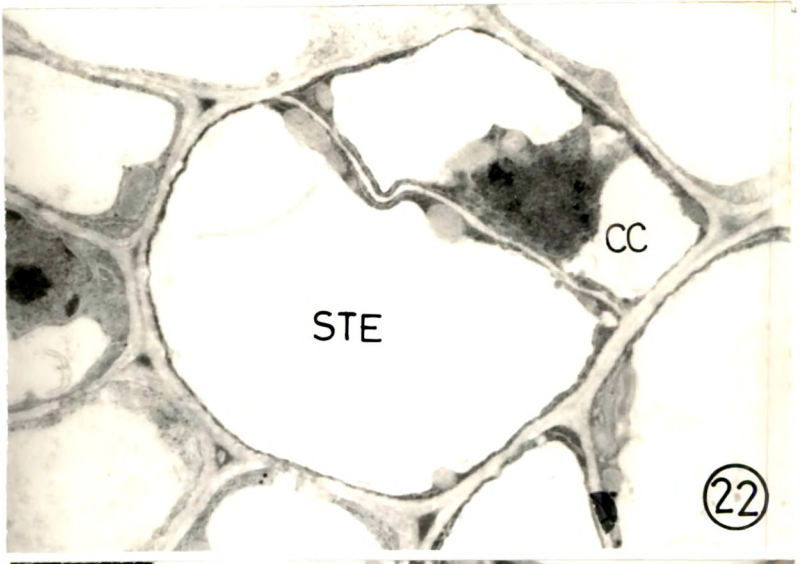
Figs.24-26. Longitudinal sections of sieve tube elements.

Fig. 24. Obliterated sieve tube element. Note the lumen is stretched (arrows). x 156.

Fig. 25. Obliterating sieve tube element showing heavy deposition of callose at the sieve plate (arrow heads) and sieve areas (arrows) x 338.

Fig. 26. Aniline blue induced fluorescence of the obliterating sieve elements. Callose is evident (arrows). x 156.


20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STE
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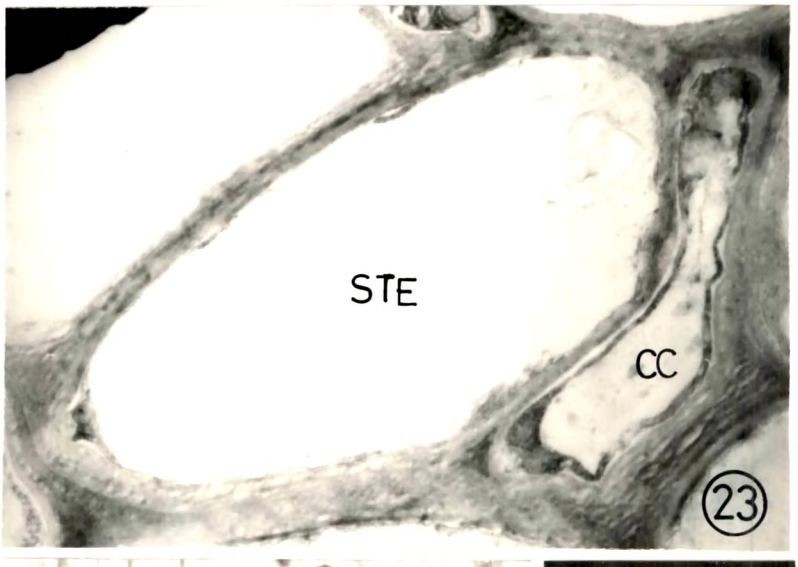
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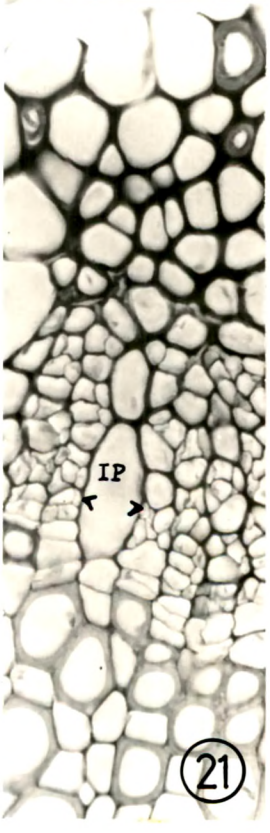
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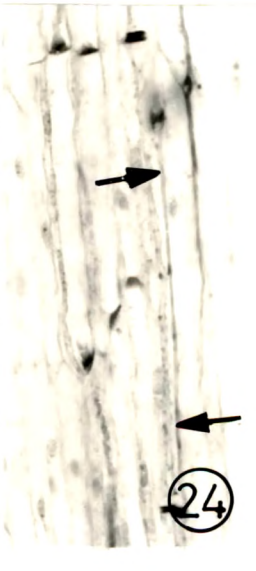
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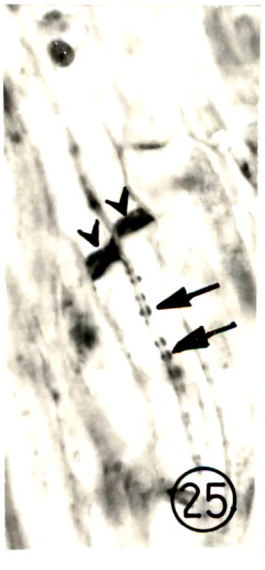
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Plate XIV

Figs. 27 and 28. Longitudinal sections of sieve tube element.

Fig. 27. Protophloem sieve tube element. x 750.

Fig. 28. Secondary sieve tube element. End wall of the secondary sieve tube element is much inclined than the protophloem sieve tube element. x 830.

Fig. 29. Transverse sections of the bark. x 125.

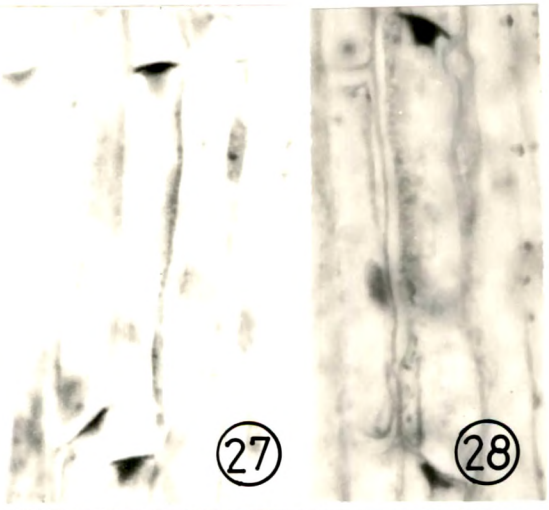
Fig. 30. A portion enlarged. Secondary phloem elements are arranged in raidal rows. x 160.

Figs. 31-33. Longitudinal sections of the sieve tube elements in the bark.

Fig. 31. A sieve tube element. x 300.

Fig. 32. A sieve tube element showing P-protein body. x 275.

Fig. 33. End wall of the sieve tube element magnified to show the slime (S) and nucleus (N). x 895.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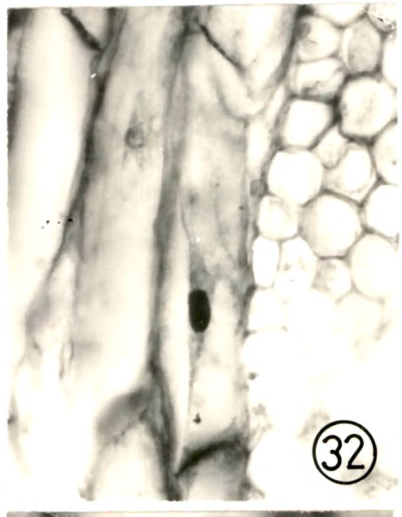
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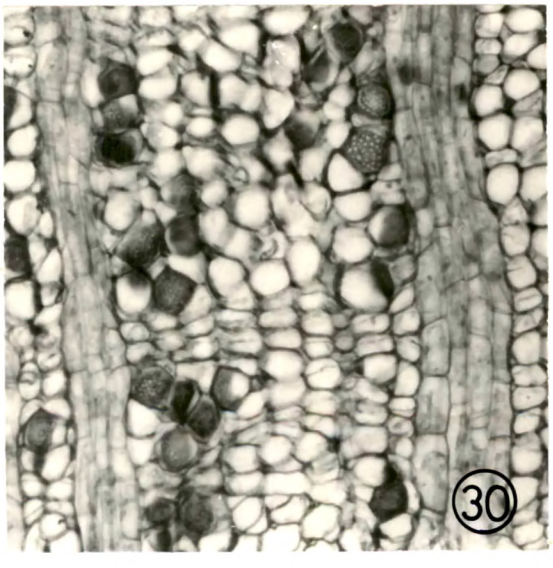
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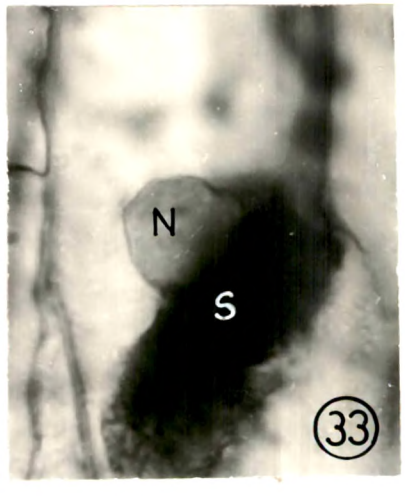

N
S
33

Plate XV

Figs. 34-39. Longitudinal sections of the wound phloem.

Fig. 34. Wounded petiole showing the wound callus (at arrow). x 21.

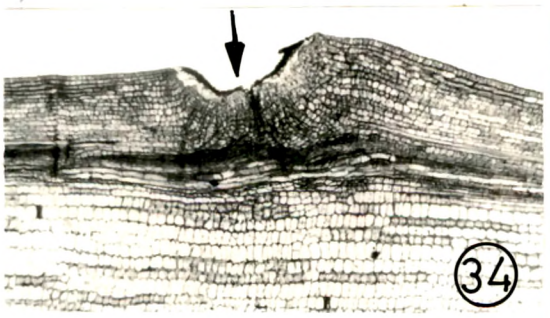
Fig. 35. Differentiation of wound sieve elements. x 380.

Fig. 36. Periclinally dividing wound cambial initial showing phragmoplasts (at arrow). x 380.

Fig. 37. A wound sieve tube element. x 1250.

Fig. 38. Newly regenerated wound sieve elements showing fluorescence of the callose (arrow head). Regenerating secondary xylem elements also show fluorescence (arrow). x 182.

Fig. 39. Wound sieve elements showing fluorescence of the callose at the sieve plate. Arrows point to the lateral sieve areas. x 1120.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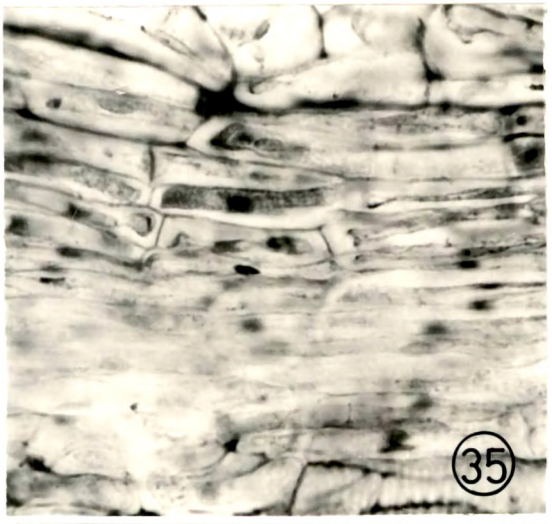
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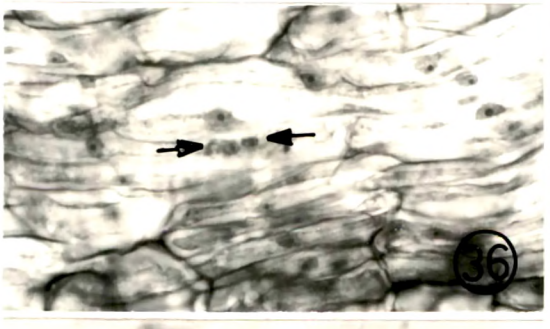
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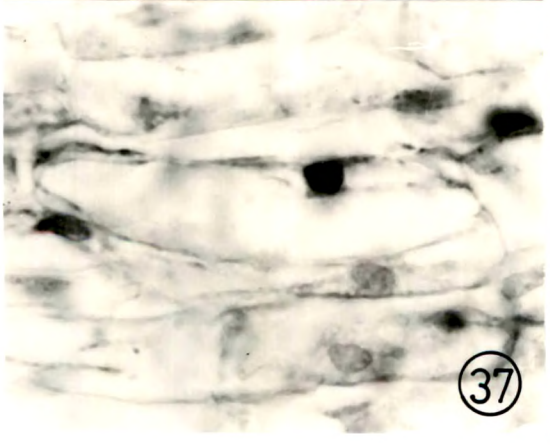
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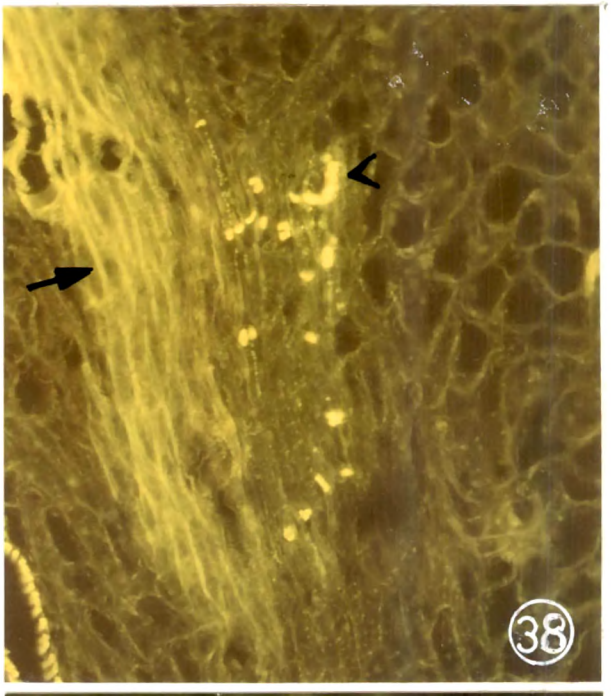
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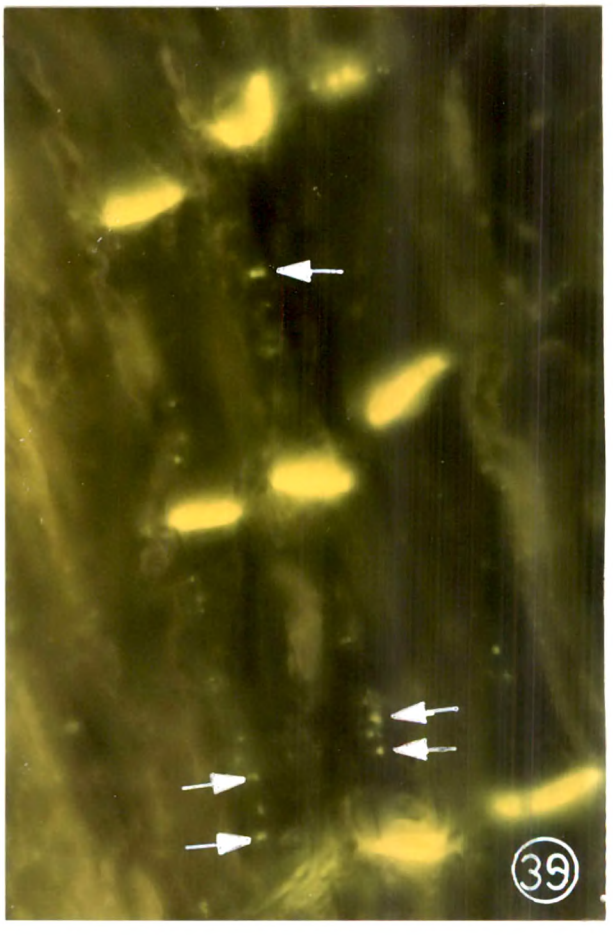
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Plate XVI

Figs.40-43. Longitudinal sections.

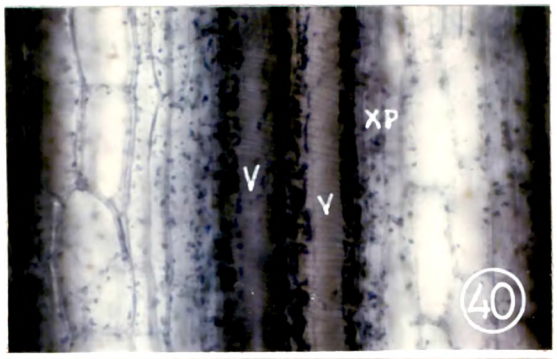
Fig. 40. Dark blue staining of the xylem parenchyma (XP) which ensheath the xylem vessels (V) in the petiole. The pith parenchyma cells do not show any staining. x 250.

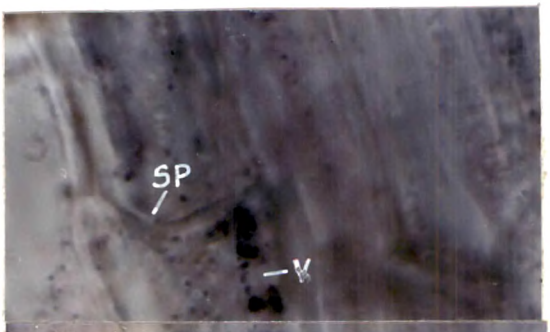
Fig. 41. Reduction of thiazolyl blue (dark blue content) in the sieve tube elements. Immature sieve elements show uniform staining (arrow) whereas only companion cells of the mature sieve tube element shows high reducing capacity (also see fig. 43). Obliterating sieve elements do not show any staining or less staining (arrow head). x 312.

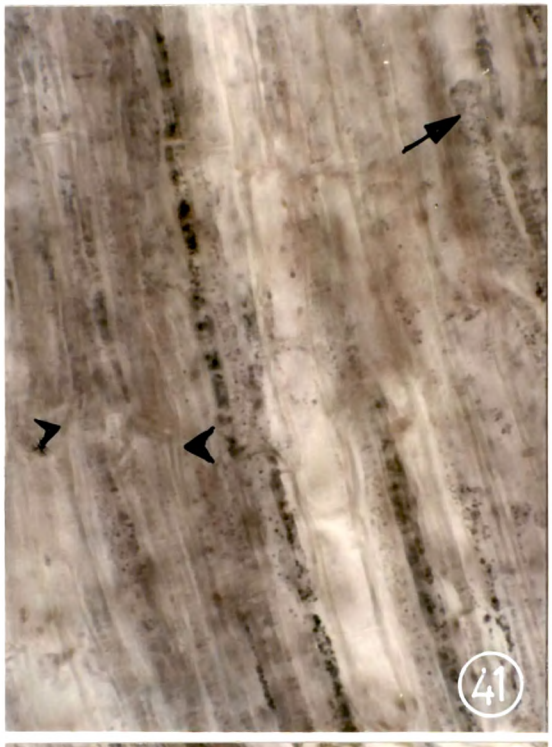
Fig. 42. Mature protophloem fibres do not show any staining whereas immature ones are stained (arrow). x 312.

Fig. 43. Detail of a sieve tube element with a companion cell with cytoplasm and vacuoles. The multiple vacuoles (V) is well demarcated by the dark blue stained cytoplasm, which are the regions of mitochondria (SP = Sieve plate) x 1000.

Fig. 44. Lower surface of a thiazolyl blue fed lamina shows the blue colouration of the veins. x 40.


XP
V
V
40


SP
V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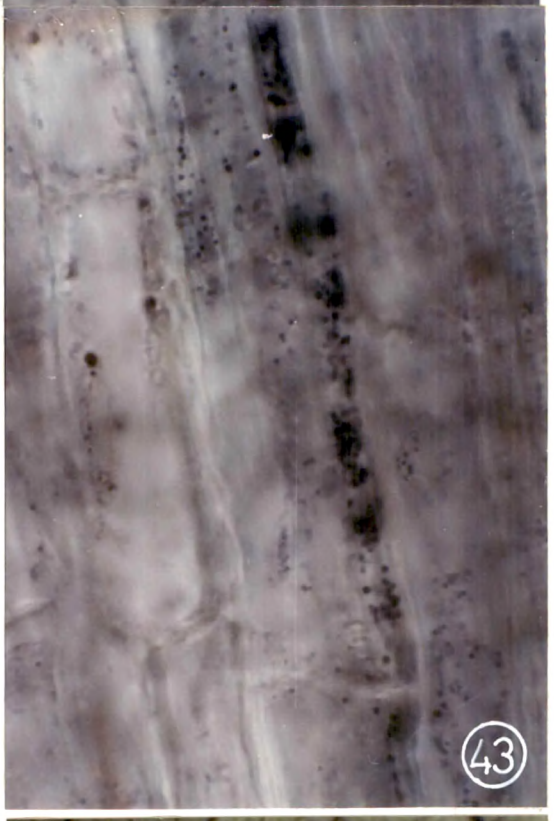
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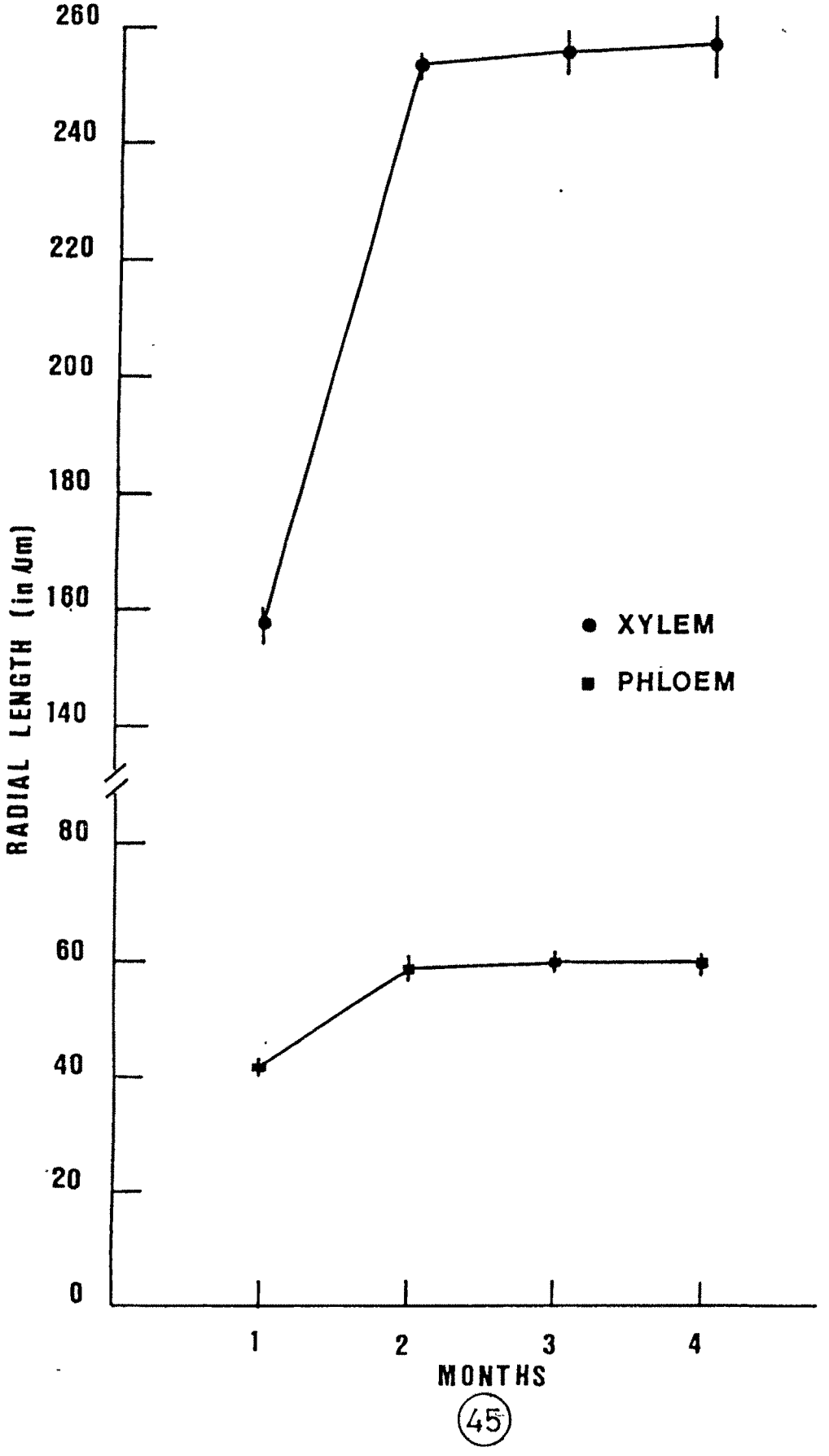
Figure 45. Radial length of the phloem and xylem (in the median transverse sections) in four month old petiole over time.


260
240
220
200
180
160
140
80
60
40
20
0
RADIAL LENGTH (in µm)
XYLEM
PHLOEM
1
2
3
4
MONTHS

SECTION IV

3.2 Salvadora persica L. (Salvadoraceae)

3.2.1 General morphology

Salvadora persica L. is a small evergreen tree with branches and drooping branchlets and glaucous bright green leaves. The trunk is straight or crooked and bark is dull grey to greenish white in colour. Leaves are opposite decussate, succulent and aromatic varying in shape from ovate to narrow lanceolate, blade 1-2 inches long with an acute apex. The tree is of periodic growth, evergreen type (Longmann and Jenik, 1974). Leaf shedding occurs long after bud opening and the life span of leaves is more than eleven months. The simple leaves are petiolate and the length of the petiole varies from 0.5 to 2 cm.

3.2.2 Internode-node-petiole continuum

3.2.2.1 Anatomy of the young node

The phyllotaxy is decussate. The young internode shows vascular strands peripherally arranged in a rectangle. It includes discrete collateral and phloic

strands. In a transverse section, approximately 80 um below the apical summit two leaf trace strands are recognized in the fourth node (fig.1). The node is unilacunar with two trace strands. They traverse obliquely upward through the cortex and then aggregate at the petiole base (figs. 1 to 8). Occasionally an additional strand is observed but during its further extension in the petiole base the strands converge to form a complex strand which further separates in the petiole.

3.2.2.2 Young petiole vasculature

Young petiole at the fourth node shows an arc shaped vasculature with incurved ends, in which 10 to 12 strands are noticed.

Each leaf is served by two trace strands, but during their traverse many strands diverge from the parent strands to form the petiole vasculature (figs. 9 to 11). Trace divergence takes place at the node. The first two lateral strands arise from the two original trace strands which traverse as median strands further in the petiole. Subsequently laterals arise from the preceding laterals as shown in figure 9. These divisions occur more or less centrifugally. Thus, outermost lateral strands away from the median strand are the youngest and smallest.

The vasculature at the base of the petiole is arc shaped with incurved ends (fig.12). It consists of 10 to 12 strands compactly arranged but separated by uniseriate interfascicular parenchyma. The arc shaped vasculature gradually flattens in the distal region, where the two lateral strands gradually separate to form the first secondary veins in the lamina (figs.13 and 14).

The number of strands remains fairly uniform in the entire petiole. Amalgamation of strands is seldom observed.

3.2.2.3 Anatomy of the mature node

The nodal structure in the mature region is comparable with that described for the young region except that secondary tissue is present. In this condition the trace strands described above have been identified by their primary xylem groups which project into the pith (fig.15). The series of figures from 16 to 22 illustrate the condition of these two trace strands in a mature node. The internodal vasculature separates to form a single gap through which the trace strands gradually move outwards towards the petiole base; successive pairs of strands become diverged from them and pass into the base of the petiole, where the strands arrange in the form of an arc (figs.23 and 24).

3.2.2.4 Vasculature in a mature petiole

In a mature petiole the vasculature is horse-shoe shaped (fig.25). It consists of about fourteen strands closely arranged and separated by interfascicular parenchyma.

The transverse section of the petiole has a more or less circular outline with upper side slightly flattened. External walls of the epidermal cells are heavily cutinized. The cortex is a broad zone of thin walled parenchyma cells having small intercellular spaces. Pericycle and endodermis are absent. The vasculature has much greater development of the secondary vascular tissue towards the rounded abaxial side. Small groups of protophloem fibres lie outside the isolated groups of crushed primary phloem cells. In the centre there is a very narrow flattened pith of small, thin walled cells. Vascular strands consist of xylem, phloem and included phloem. Xylem includes fibres and vessels. The structure of the phloem is described in a later section.

3.2.3. Leaf architecture

The basic axis of orientation is basal, the curvature of leaf elements is convex. Leaf organization is simple, consisting of a single lamina (fig.26).

Basilaminar colletors are present. Petiole is normal without noticeable thickenings or other processes.

Venation is pinnate, and brochidodromous. A single primary vein serving as the origin for the higher order venation. The primary vein is weak with a straight course. The angle of divergence of secondary veins is acute, narrow; the upper secondary veins are more obtuse than lower. Secondary veins are fine to hair-like, proportionately narrow in relation to the primary and tertiary vein order. Its course is curved, bending in an arc. Tertiary veins anastomose with other tertiary veins or with secondary veins forming a random reticulate network (fig.27). Higher order venation forms a reticulum in which vein orders cannot be distinguished. Marginal ultimate venation is incomplete, freely ending veinlets are present directly adjacent to the margin. Veinlets are branched twice or more. Areole development is imperfect with meshes of irregular shape and size (fig.28). The arrangement of areoles is random and their shape is polygonal or irregular.

Plate XVII

Figs. 1-8.	Transections of a young node. All x 100.
Fig. 1.	Two trace strands related to unilacunar node (arrows).
Fig. 2.	Trace strand gradually move towards the cortex region.
Figs. 3-5.	Identity of the individual strands become indiscrete.
Figs. 6-8.	The vascular strands gradually separates from the internodal vasculature and arrange in the form of an arc at the basal region of the petiole.

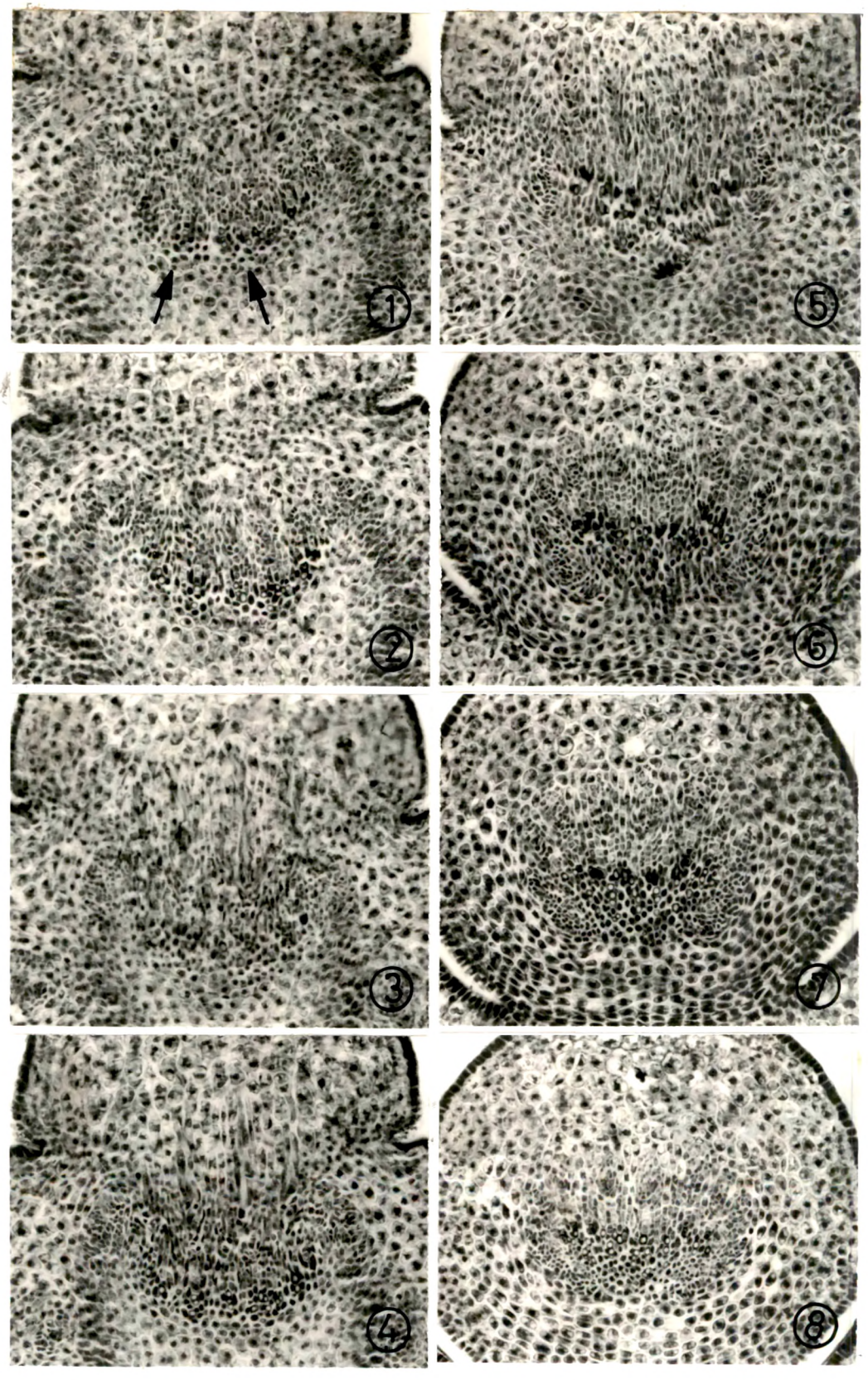

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Plate XVIII

Fig. 9. Two dimensional view of the internode-node -petiole continuum.

Fig. 10. Lactic acid cleared internode with a petiole. It shows two trace strands (arrows) and petiolar strands (arrow head). x 8.

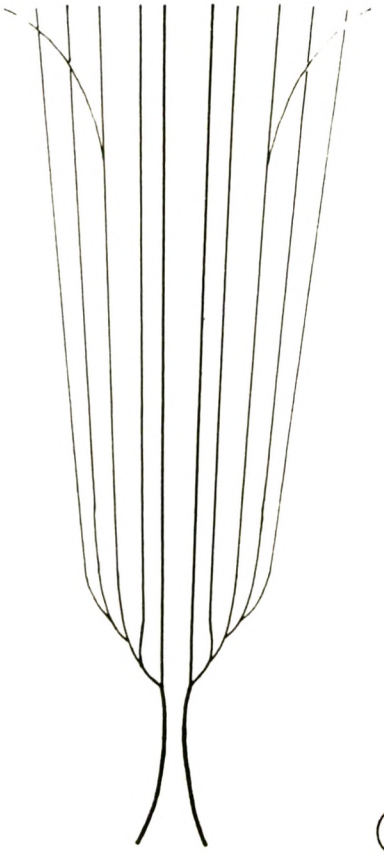
Figs. 11-14 Transections. All x 112.

Fig. 11. Node. Arrows indicate the two leaf trace strands.

Fig. 12. Base of the petiole.

Fig. 13. About two-third of the distance from the base (Arrows indicate one of the first secondary vein).

Fig. 14. Distal region.



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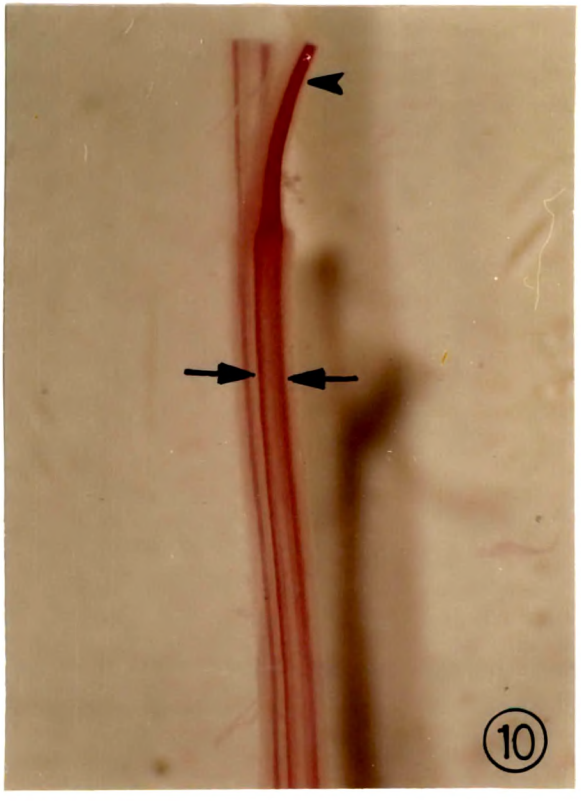

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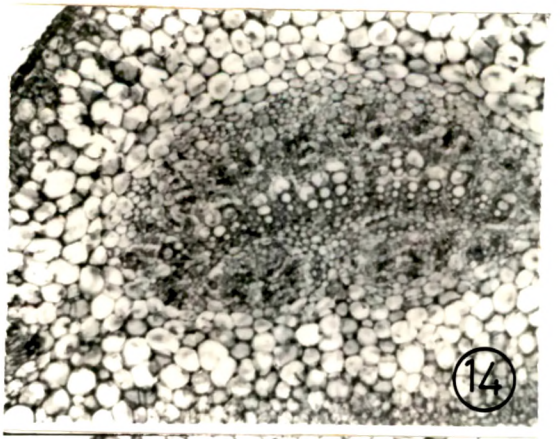

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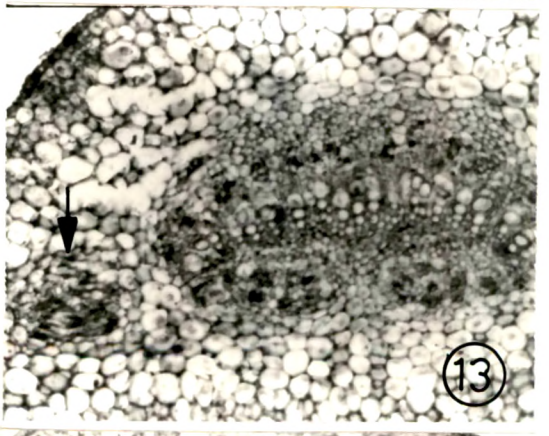

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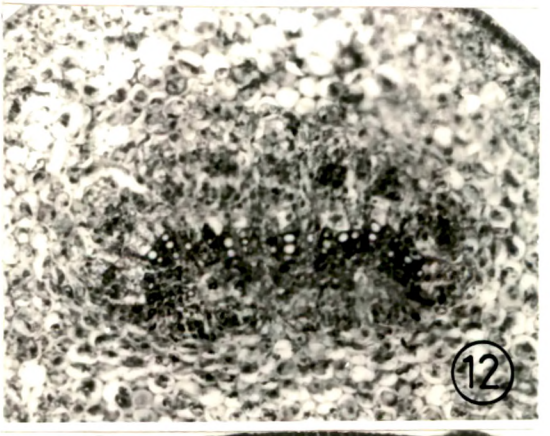

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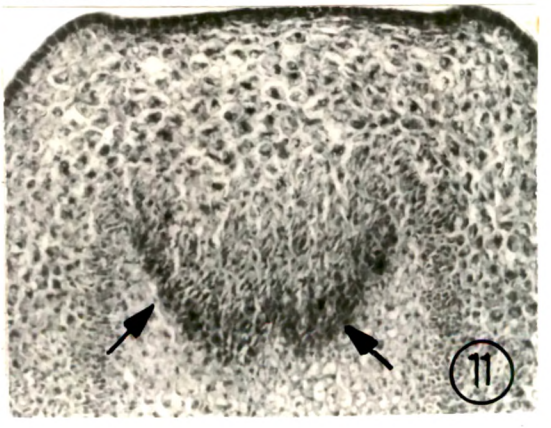

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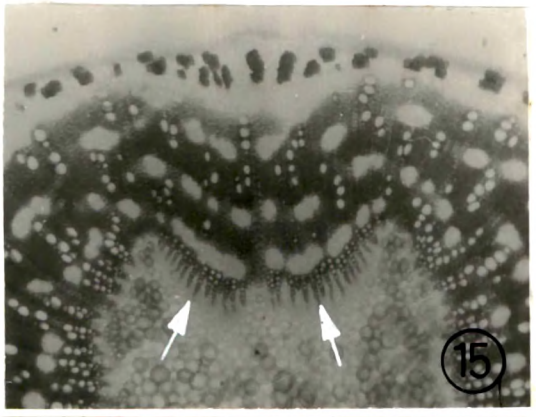
Plate XIX

Figs. 15-22. Transections of a mature node. Lactic acid cleared sections. All x 15.

Fig. 15. Two trace strands. Arrows indicate the protoxylem groups of the two trace strands.

Figs. 16-22. Trace strands gradually separate from the internodal vasculature leaving a gap. The strands converge towards the cortex and more obliquely upwards as a strand. During its further development into the petiole many strands bifurcate from it to form the petiole vasculature.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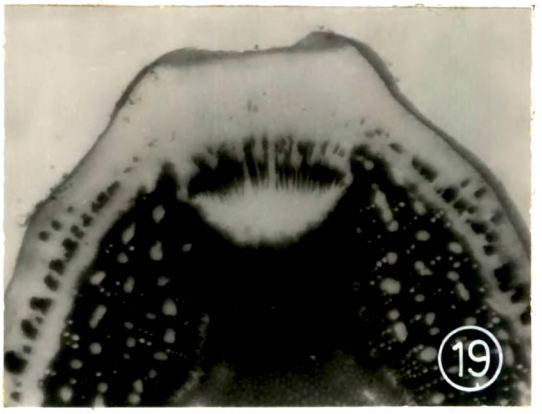
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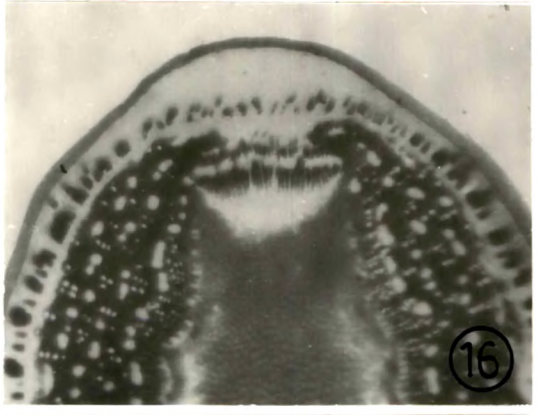
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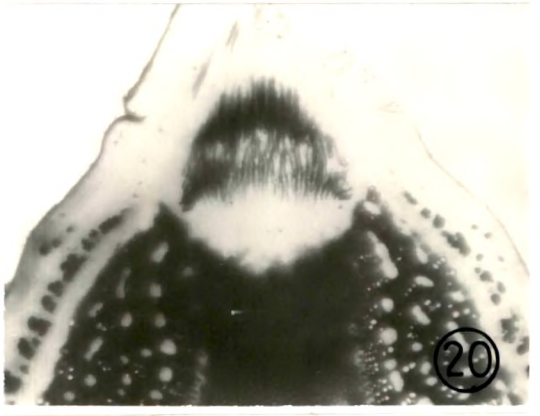
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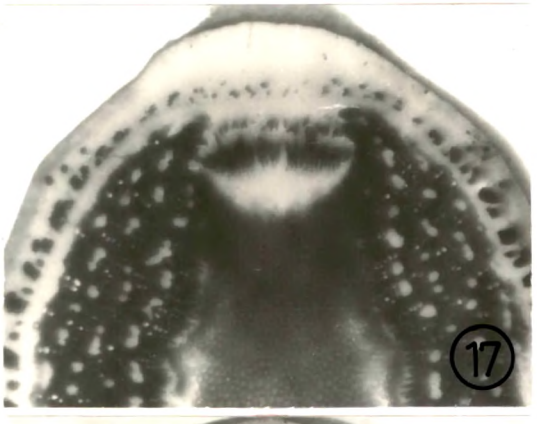
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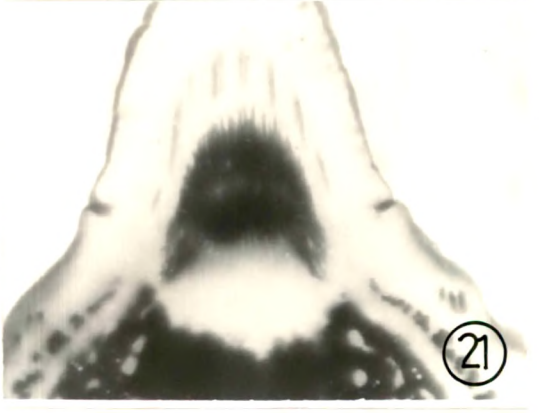
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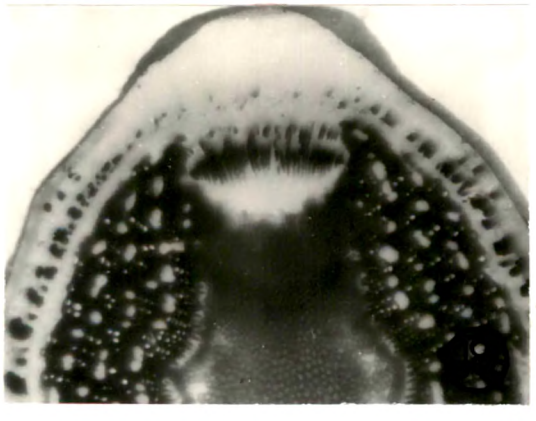

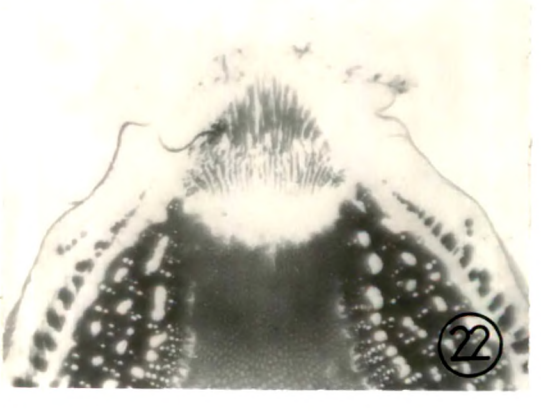
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Plate XX

Figs. 23 and 24. Cleared nodes.

Fig. 23. Basal region of the petiole dhowing arc shaped vasculature (arrow). x 14.

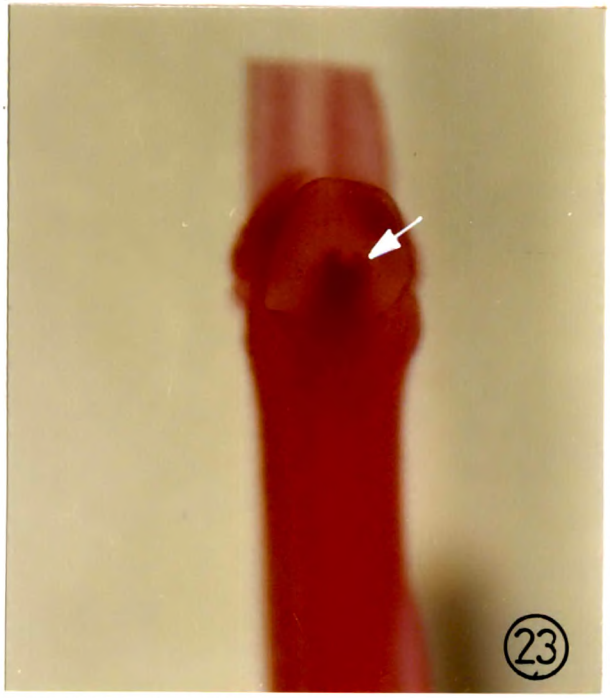
Fig. 24. Mature nodal region shows the trace before its entry into the petiole (LG = Leaf gap). x 22.

Fig. 25. Transection of a mature petiole showing the vasculature (INP = Included phloem; PF = protophloem fibres). x 136.

Fig. 26. Leaf clearing showing the vascularization of the lamina (M = Mid vein, S = Secondary vein; T = Tertiary vein). x 2.

Fig. 27. Details of leaf clearing showing mostly minor veins. x 9.

Fig. 28. Enlarged portion of a cleared lamina showing areoles of irregular size. Free vein endings are also seen. x 27.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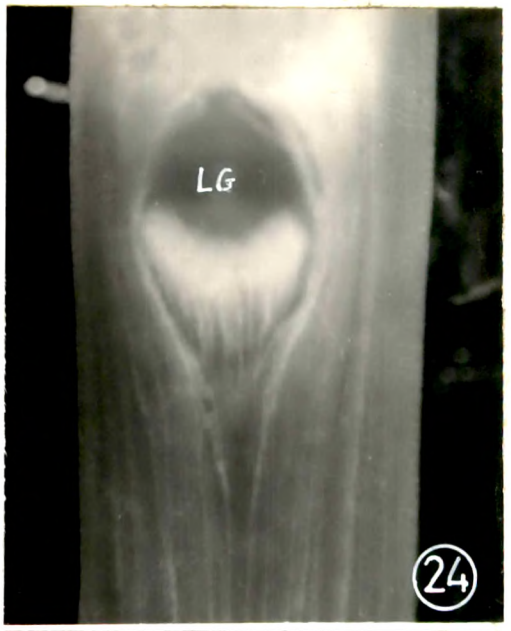
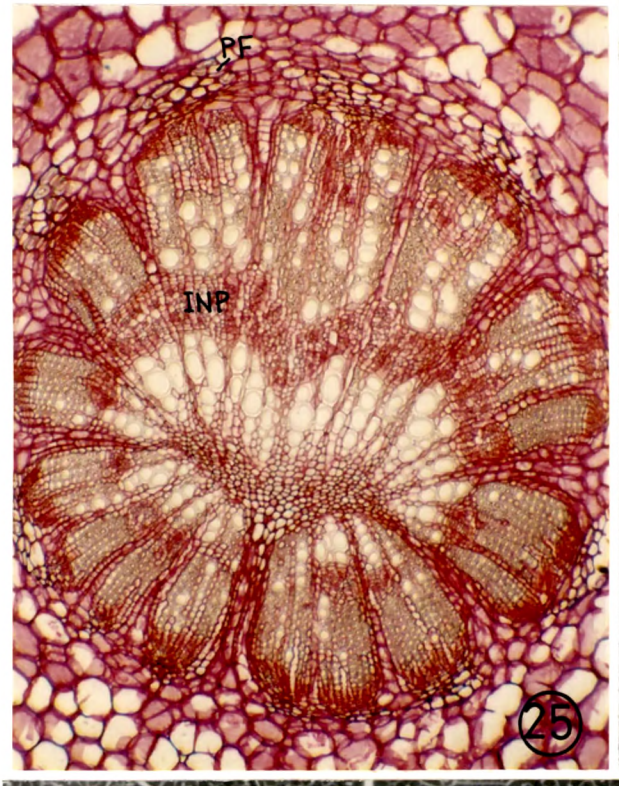
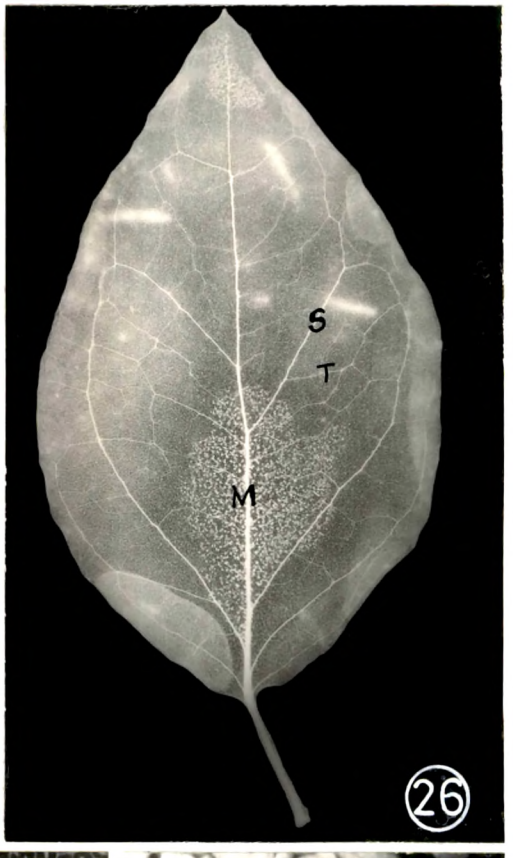
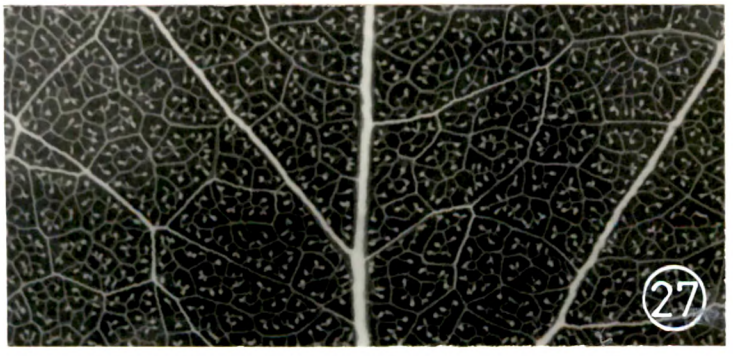

LG
24


PF
INP
25


S
T
M
26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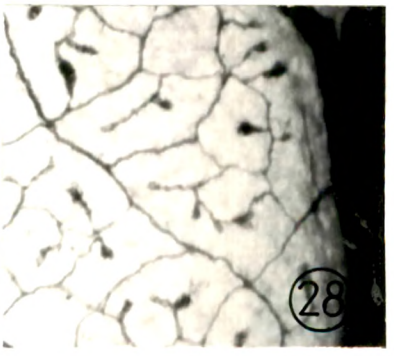
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SECTION V

3.2.4 Primary vascular differentiation in the petiole

The study of vascular differentiation in the petiole is carried out by the examination of both transverse and longitudinal serial sections of the shoot tip. Leaves are situated in pairs in decussate phyllotaxy.

3.2.4.1 Transverse course of differentiation

The procambial strands could not be detected in relation to the youngest primordium at the first node. But an actively dividing locus of cells is evident at the basal region which may be considered as an advancing front of the procambial strands. At a level approximately 80 um below the apical summit the primordia at the second node are each served by two recognizable procambial trace strands. These two strands are visible in the vascular meristematic arc as loci of actively dividing cells (fig. 1). It should be noted that, as mentioned earlier, during its traverse into the petiole the orientation of the arc reverses. The primordium at the third node is served by four recognizable strands alongwith intervening vascular meristem (fig. 2). The developing petioles at the fourth (fig. 3) and fifth nodes have eight and ten strands

respectively. Fourteen strands are found to be a constant number in the mature petiole.

Procambial strands of developing leaves are more advanced in development near their levels of origin than at higher levels in the petiole. For example, in the second node the two trace strands are poorly defined relative to the vascular meristem present in the distal region (fig. 4). However, they are more differentiated at the basal region (see fig. 1). This developmental trend is also most conspicuous in other petiolar strands.

3.2.4.2 Longitudinal course of differentiation

The terminal meristem, i.e. the shoot apex is a high, domeshaped mound and leaf primordia are initiated on its slopping flanks (fig. 5). The shoot apex has tunica-corpus organization - a single layer of tunica overlying a mass of corpus cells. The corpus shows three main zones namely, central meristem, peripheral meristem and pith meristem (fig. 6). Cells of the pith begin vacuolation very close to the summit of the apex and enlarge much more rapidly than do the cells that surround them peripherally. It is from the peripheral meristem that leaf primordia arise.

The basal region of the primordium at the first node shows darkly stained vascular meristematic region. The procambial strands are not yet discretely established in the primordium.. In the second node, where the primordium is elongating the procambium is identified both by the elongated shape of its constituent cells and by their intense affinity for staining. Procambium is recognizable by longitudinal divisions that occur with greater frequency than surrounding cells of the vascular meristem with the result that procambial cells appear elongated, that is, they are longer than they are wide. Intervening vascular meristematic cells are evident in longitudinal sections only by their less affinity for staining.

3.2.5 Differentiation of procambium in the petiole

Serial sections of a shoot tip were used to study the vascular continuity between the internode, node and petiole. The leaf primordium at the second node has a recognizable petiole. Young developmental stages of the petiole in Salvadora are categorized into five stages based on their length. They are placed at the second, third, fourth, sixth and seventh nodes respectively from the shoot apex.

As described for Crataeva earlier, the procambium in

the early stages of petiole elongation is identified by its greater affinity for stains, and less orderly arrangement. Groups of procambial cells can be recognized by their dense staining and small size relative to the adjacent cells in transverse plane. Intervening cells of the vascular meristem are also densely stained but because of their greater mitotic activity procambial strands are more prominent.

In the petiole of Salvadora at the basal region of the second node two procambial strands develop acropetally. These two strands are visible in the vascular meristem as two actively dividing loci identified on the basis of cytoplasmic density and cell orientation (fig. 7). During its further traverse into the petiole the orientation of the arc reverses and the median portion becomes the dorsal one. Out of the two procambial strands one possess a sieve tube element (fig. 8). Towards the distal part of the petiole, the vascular region is evident as a darkly stained meristematic zone.

Following the primordium establishment lateral strands differentiate within the petiole base from the two procambial strands and develop acropetally. These lateral strands diverge from the two procambial traces. A 1 mm long petiole at the third node shows two median and

two lateral strands interspersed with interfascicular vascular meristem. Because of their earlier appearance in the primordium, the median strands are more developed than their corresponding lateral strands. Each of these median strands possess 4-5 sieve tube elements and 1-3 protoxylem elements (fig.2). The protophloem and protoxylem regions are widely separated by differentiating procambial cells. New procambial strands arise in the vascular meristematic arc in a centrifugal manner.

As the petiole advances upward from the node, the procambial strands at the basal region would gradually appear more discrete and segregated. Parenchyma cells in the pith region become vacuolate. In the basal region of the petiole at the fourth node about eight strands are observed. Each of these strands is separated by densely stained, usually one cell wide, radially elongated interfascicular vascular meristematic cells mostly differentiating as interfascicular or interspersed parenchyma (fig.9). More protophloem sieve tube elements differentiate in the median and lateral strands. As noticed in the earlier stage, the procambial region between protophloem and protoxylem is densely stained and its cells are more or less radially arranged. Each radially elongated strand is 3-4 cell wide. Here, protoxylem elements show more pronounced radial

seriation. Longitudinally the procambial cells remain as somewhat elongated and rectangular, with a large round nucleus and dense cytoplasm.

As the development of the petiole progresses further the strands become more discrete and segregated. The intervening vascular meristematic cells between the adjacent strands gradually differentiate as interfascicular parenchyma. Pith parenchyma cells and cortical parenchyma cells appear vacuolated. The basal region of the petiole at the sixth node shows 8-10 strands arranged in the form of an arc with incurved ends. The first signs of obliteration of protophloem sieve elements were noticed at this stage. The densely stained meristematic procambial region gradually transforms into less dense region in which one or two isolated, periclinally dividing procambial cells are discernible adjacent to the differentiating metaxylem vessel elements in the median strands (fig.10).

Although late formed protoxylem elements are indistinct from early formed metaxylem elements, their discernment can arbitrarily be made based on their relative appearance in the strands, their size and position within the strands. The advance stage of the vascular meristem from which the metaxylem differentiates

is, however, considered as procambium. The 2-3 layered procambium is continuous in the median strand (fig.11). Between two adjacent strands, their continuity is disrupted by the uniseriate interfascicular parenchyma. Occasionally in some strands interspersed parenchyma is also observed. The procambial cells at this stage are more vacuolated and hence density of staining appears decreased.

3.2.6 Xylem development

Protoxylem elements differentiate relatively early. Of the four recognizable strands serving the primordium at the third node, two median strands possess protoxylem elements (fig.2). The first protoxylem elements differentiate at the approximate level of the leaf primordium base and towards the abaxial side of the primordium. From here, protoxylem differentiates acropetally in the incipient leaf mid rib. Protoxylem elements were first observed in two median procambial bundles and then they developed in the laterals.

The sequence of maturation of protoxylem elements is centrifugal. The first formed protoxylem elements become stretched and subsequently obliterate in the later development of the petiole. This development is apparent

in the median strands because their protoxylem elements mature earlier and, therefore, are subjected to greater elongation and compression than those of the lateral strands.

Both protoxylem and metaxylem elements show radial arrangement. Radial seriation of procambium is more evident during the differentiation of metaxylem where the frequent periclinal divisions of the procambial cells occur.

Although radial seriation of differentiating procambial region is observed during the protoxylem development the micromorphology of procambium becomes more distinct during the metaxylem differentiation. Metaxylem differentiation is first noticed in a 3 mm long petiole but it should be pointed out that differentiation of late protoxylem and early metaxylem may overlap in time.

3.2.7 Transition of procambium to cambium

In Salvadora the initiation of secondary growth in petiole is correlated with the cessation of its elongation. Certain anatomical features which accompany the initiation of secondary growth are also considered as complementary criteria to determine its beginning.

The procambium is a homogeneous tissue in its early development (fig.12). Its cells are elongated with transverse end walls and have a round nucleus and densely stained cytoplasm. The first protoxylem elements with annular wall sculpturing develop during early phase of the petiole elongation. They function for a very short time and then are passively stretched during subsequent petiole elongation. They are soon replaced by the metaxylem elements that develop later. As the petiole elongates further the procambial zone in each strand increases both radially and tangentially, the procambial cells become further elongated and their end walls tapered. The apical intrusive growth of the procambial cells becomes prominent during the late stages of petiole elongation and they gradually attain the fusiform nature (figs. 13 and 14). Their cytoplasm appears less dense because of increased vacuolation. The nucleus also gradually changes from round to spindle shaped (figs. 15 and 16).

The aforementioned changes take place gradually during the late phase of elongation. However, the characteristics attributed to the normal vascular cambium are evident after the transition stage. The active periclinal divisions in the procambial cells are followed by repeated anticlinal divisions which lead to the

appearance of typical horizontal rows or storied fusiform initials. Although anticlinal divisions in the procambial cells occur in the late stages, the frequent yet irregular transverse divisions prevent the early establishment of storeyed pattern.

Concomitant with these changes other developmental features also appear signalling the transition. The characteristic beaded nature of the wall of fusiform initials appears. The lignification of walls of protophloem fibres and xylem parenchyma occurs as they show birefringence in the polarized light (figs. 17-19). Since the included phloem develops only after the cessation of petiole elongation, this feature can also be considered as a significant characteristic by which the initiation of secondary growth is identified.

Dimensional changes of the procambium and cambium are given in Table V.

3.2.8 Vascular cambium and its activity

The vascular cambium is observed only in the strands and no interfascicular cambium is present. It is 2-3 layered and storied. Fusiform cambial cells are tangentially tapered. Occasionally transverse divisions

also occur in them. The nucleus is spindle shaped and cytoplasm is lightly stained. Rays are absent (fig. 20).

Initiation of secondary growth in the petiole is accompanied by the wall lignification of protophloem fibres and xylem parenchyma, and by the development of xylary fibres and included phloem. The vascular cambium shows anomalous behaviour during the secondary growth. Soon after the secondary growth commences, the outer xylem presents a more or less indented outline, which is due to the fact that at certain loci the cambium cuts off towards inside some tangentially elongated groups of thin-walled derivatives. The cambium at this locus presents a normal storied structure. They are in perfect radial rows and differ from the normal cambial cells only in having a large radial diameter. Later the cambium resumes its normal activity so that the groups of thin walled differentiating phloem cells becomes enclosed between the thick-walled cells of the previous and newly formed xylem (figs.21 and 23). As the growth proceeds some of the central cells in the islands of included phloem differentiate into one or more groups of sieve tubes and companion cells but the peripheral cells still remain meristematic and often undergo some periclinal divisions (figs.22 and 24). In some earlier formed islands of the included phloem these meristematic cells appear in segments on any one side, or more than one

sides of the island or even all around it.

3.2.9 Vascular cambium in the stem

The vascular cambium in the stem of Salvadora is storied comprising two systems of cells; fusiform initials and ray initials. Fusiform initials are long and arranged in definite vertical rows. Ray initials are responsible for the production of multiseriate rays. The cambial initials and their immediate derivatives form cambial zone which consists of undifferentiated cells arranged in radial rows (fig.25). The cambial zone is about 6 to 8 layers thick. Fusiform initials as seen in tangential sections are with tapering ends and uniform in size. They are mostly uninucleate. The nucleus is spindle shaped and the cytoplasm is lightly stained. Radial walls have a beaded appearance (fig.26).

Comparative features of the vascular cambium in the petiole and stem is given in table VI.

Table V : Dimensions of the procambium and cambium during the development of the petiole in Salvadora. Dimensions are in μm. Value in brackets shows the range.

	Length		Width	
Length of the petiole in mm	Procambium	Cambium	Procambium	Cambium
1.6	19.56 (15.7 - 25.12)	-	4.38 (3.14 - 6.28)	-
3.0	29.52 (23.55 - 32.97)	-	5.83 (4.71 - 6.28)	-
8.0	53.0 (40.82-59.66)	-	5.83 (4.71 - 6.28)	-
12.0	-	66.2 (51.81 - 97.34)		7.06 (6.28 - 7.85)

Table VI : Comparative features of the vascular cambium of the petiole and stem (July collection)

	Petiole	Stem
Cell types	Fusiform initials	Fusiform initials and ray initials
Arrangement	Storied	Storied
Fusiform initials		
Length μm	70.65	127.65
Width μm	7.06	15.03
No.of nucleus	Uninucleate	Uninucleate
Radial diameter μm	3.14	6.90
No. of layers of cambium	2 to 4	5 to 6

Plate XXI

Figs. 1-4. Transections of the shoot tip.

Fig. 1. Second node from the shoot apex showing basal region of the leaf primordia. Arrows point to the two trace strands. x 217.

Fig. 2. Two median strands in the basal region of the petiole at the third node showing protophloem sieve elements (arrow) and protoxylem elements (arrow head) (PC = procambium). x 217.

Fig. 3. Basal region of the petiole at the fourth node. A portion enlarged to show the petiole vasculature. Note the lightly stained interfascicular region (arrow) and procambial cells. See Fig.9. enlarged view. x 217.

Fig. 4. Distal region of the leaf primordium at the second node. Densely stained actively dividing zone (arrow) is evident in the centre of the leaf primordium axis. x 217.

Figs. 5 and 6. Longitudinal sections of the shoot apex.

Fig. 5. Apex with a pair of leaf primordia at the second node. Darkly stained region is the acropetally developing procambial region. x 153.

Fig. 6. Shoot apex enlarged to show the tunica (T), rib meristem (RM) and flank meristem (FM). x 300.

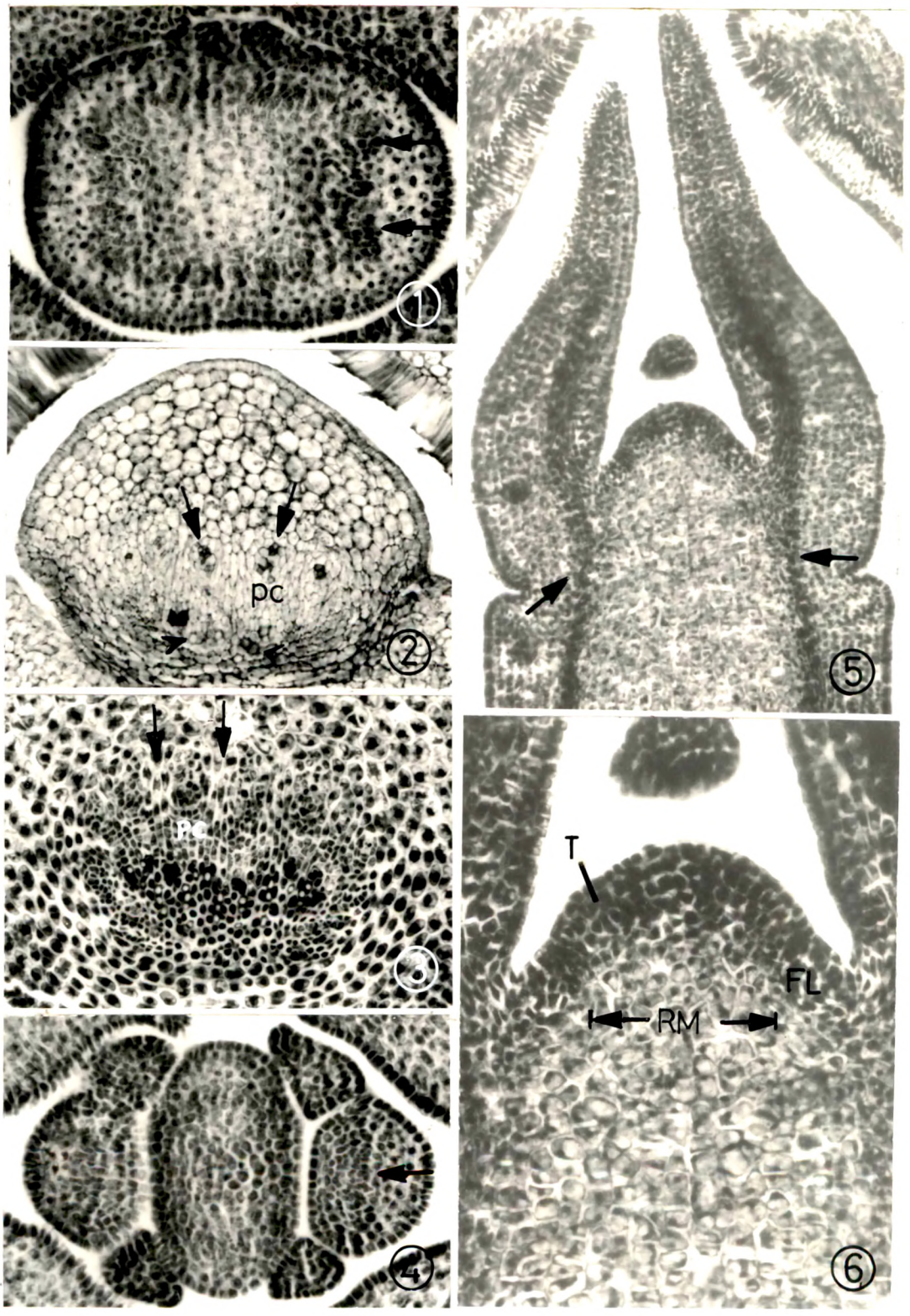

1
pc
2
3
4
5
T
FL
RM
6

Plate XXII

Figs. 7-11. Transections.

Fig. 7. Basal region of the second node. Note the differential staining (arrows) of the procambial strands. x 253.

Fig. 8. A portion of the procambial strand to show the differentiation of first sieve tube element (arrow). x 1125.

Fig. 9. A portion of the vasculature from the petiole at the fourth node enlarged. Densely stained procambial cells (PC) and protoxylem elements (PX) are radially arranged. x 350.

Fig. 10. A portion of the vasculature from the petiole at the sixth node. Procambial region (PC) is lightly stained. x 400.

Fig. 11. Late stage of procambium in the petiole showing pronounced radial seriation. x 450.

Figs. 12-16. Tangential longitudinal sections of the procambium.

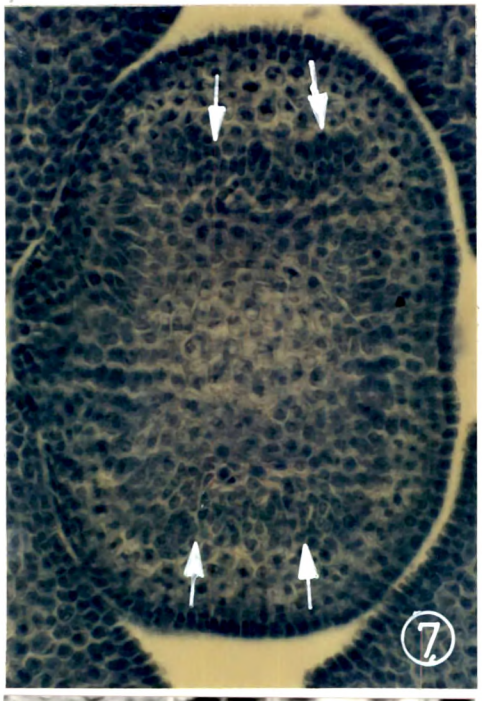
Fig. 12. Early stage cells are homogeneous. x 400.

Fig. 13. Late stage. Note the procambial cells with fusiform end walls (arrows). x 400.

Fig. 14. A periclinally dividing procambial cell showing phragmoplasts (arrows). x 625.

Fig. 15. Round nucleus of of a procambial cell in the early stage of development. x 1410.

Fig. 16. Spindle shaped nucleus in the late stage of development. x 1410.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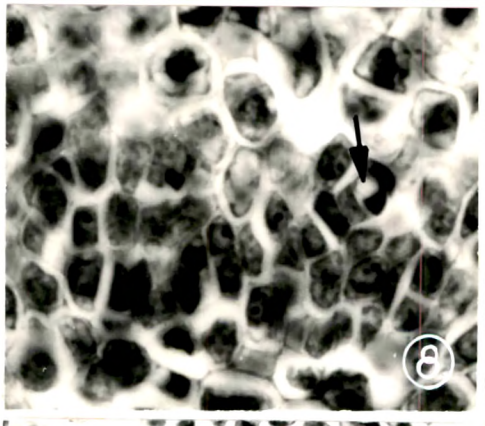
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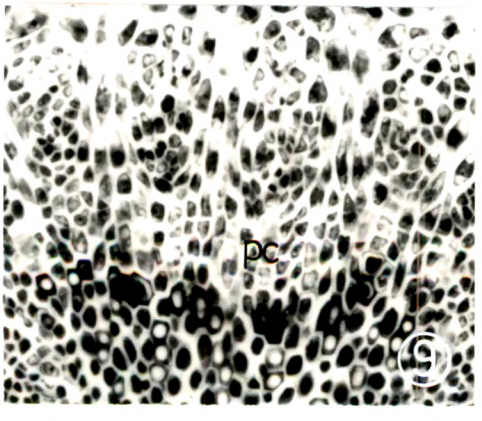
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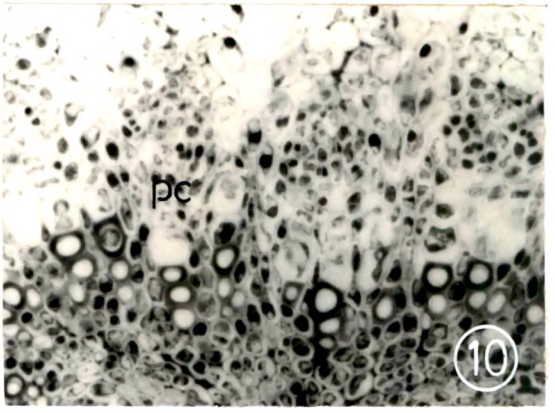
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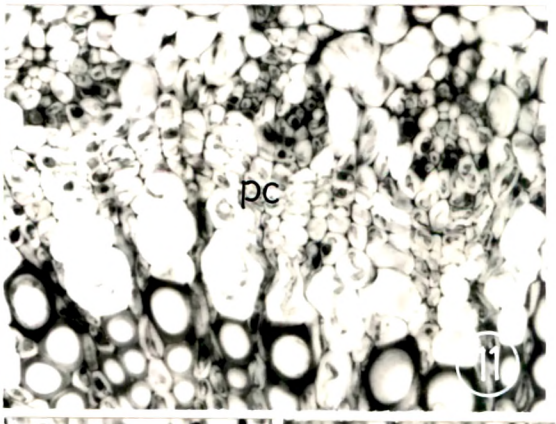
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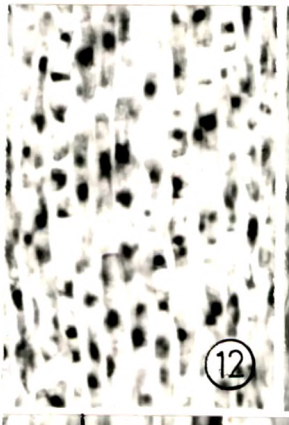
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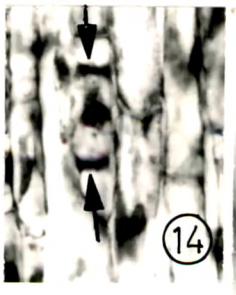
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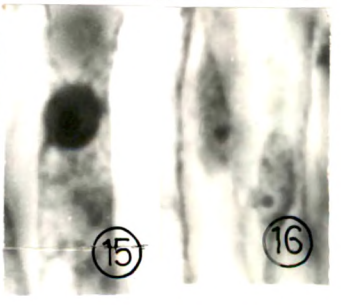
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Plate XXIII

Figs.17-19. Polarized light micrographs of the petiole vasculature.

Fig. 17. A young petiole. Only protoxylem and metaxylem elements show birefringence. x 78.

Fig. 18. Two month old petiole. Secondary xylem (SX) and protophloem fibres (arrow) show birefringence. x 78.

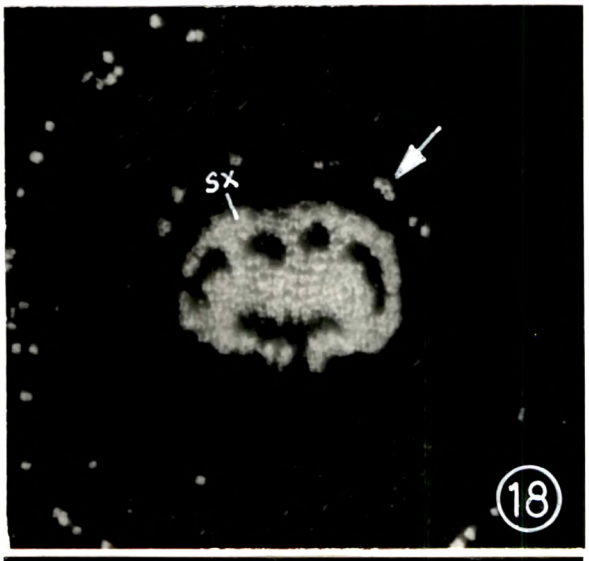
Fig. 19. Ten month old petiole. Secondary growth has added many secondary xylem elements (SX). Interfascicular region does not show any birefringence (INP = Included phloem). x 78.

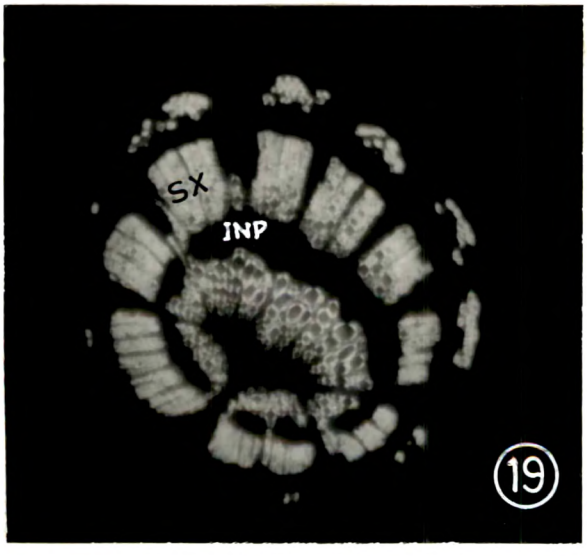
Fig. 20. Tangential longitudinal section of cambium in the petiole. Note the storied arrangement of fusiform initials (FI). x 375.

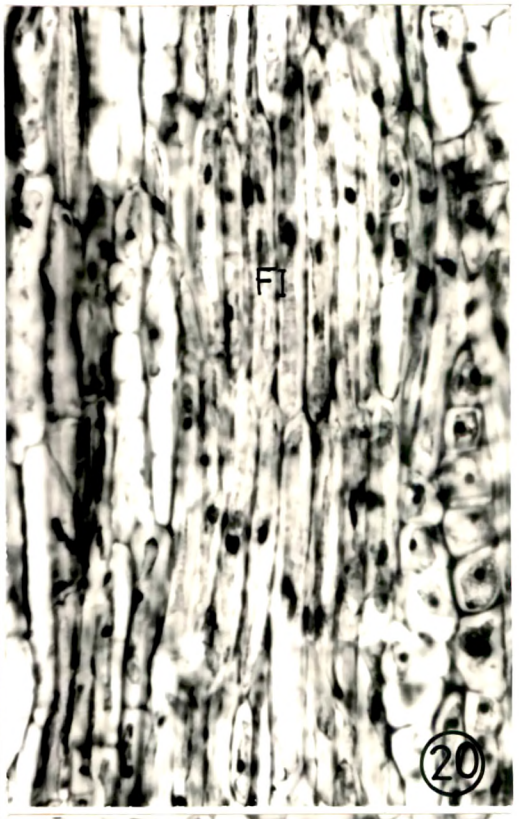
Fig. 21. A portion of the petiole vasculature showing developing included phloem (INP) and secondary xylem (SX). (Mx = metaxylem).x 225.

Fig. 22. A portion of mature petiole vasculature enlarged showing a patch of included phloem (INP). The peripheral cells are cambial in nature. x 288.


17


SX
18


SX
INP
19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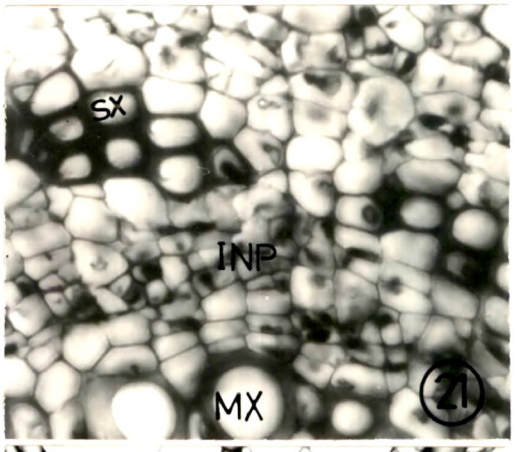

SX
INP
MX
21

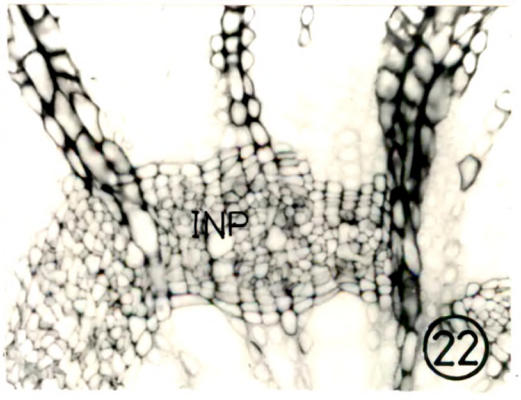

INP
22

Plate XXIV

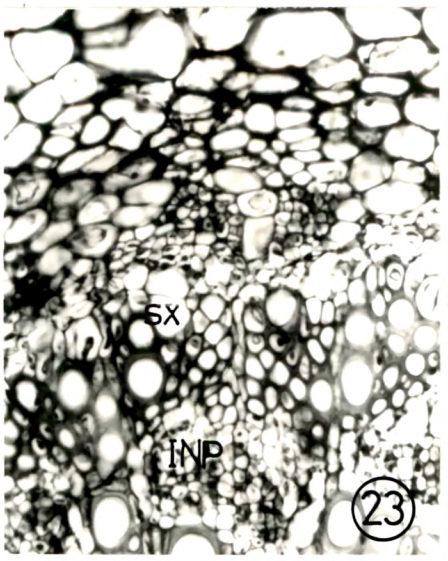
Figs. 23 and 24. Transections of mature petiole. A portion enlarged.

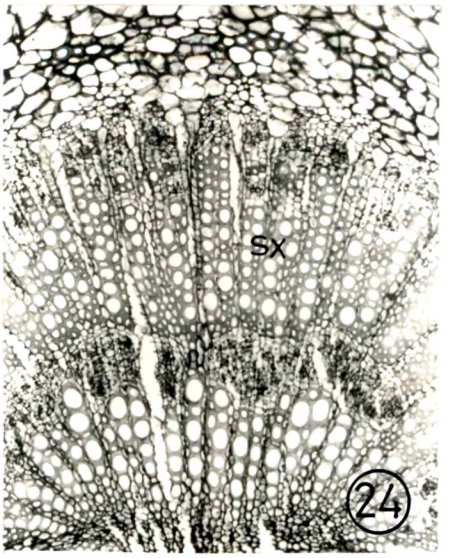
Fig. 23. A portion of the vasculature. Developing secondary xylem elements (SX) outlying the Included phloem elements (INP). x 187.

Fig. 24. A portion of the vasculature. Radial length of the secondary phloem is more or less the same whereas secondary xylem shows considerable increase. x 168.

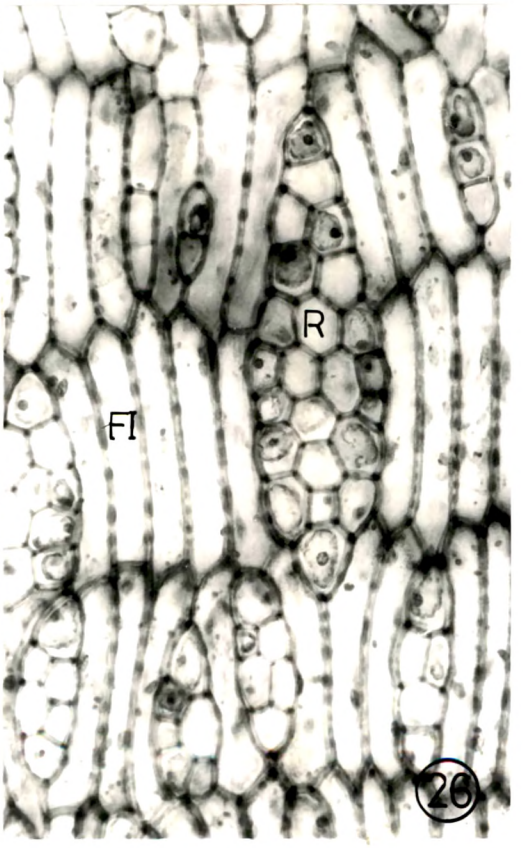
Fig. 25. Transection of the bark. A portion enlarged. Cambial zone (CZ) flanking on both sides by phloem. x 160.

Fig. 26. Tangential longitudinal section of cambium in the stem. (FI = fusiform initials; R=Rays). x 187.


Sx
INP
23


Sx
24


CZ
25


R
FI
26

SECTION VI

3.2.10 Structure and development of the phloem

It has been shown earlier that certain developmental features of vascularization in the petiole are related with its elongation, cessation and maturation. After the leaf primordium attains about 1 mm in length then some of the cells in the procambial strand differentiate into primary phloem and primary xylem.

The first sieve tube element was observed in one of the median strands in the basal region of the leaf primordium at the second node (fig.8, Section V). It differentiated along the outer periphery of the procambial strand leaving a group of parenchyma cells external to it. These cells are the precursors of protophloem fibres. The development of the phloem precedes that of the xylem. The vascular differentiation first begins in one of the median strands, then it follows in the other median one and then in the lateral strands. The differentiation of the first protophloem elements occurs centripetally.

The delimitation of late formed protophloem from early formed metaphloem is found difficult in Salvadora

because of the late appearance of radial seriation in procambial cells and relatively early appearance of metaxylem. A distinct formation of metacambium is noticed only in the late stage of elongation.

The first formed protophloem sieve elements are easily discernible by their empty lumen and thick cell wall which sharply contrast against the parenchyma cells adjacent to them (figs. 2 and 8, Section V). They occur in groups and are comparatively longer than their associated parenchyma cells. Companion cells are rarely observed. The sieve plate is simple on the transverse end wall. The sieve areas on the lateral walls are aggregate.

The procambium presents a more pronounced radial seriation of cells during the late stage of petiole elongation. Late formed metaphloem sieve tube elements are generally associated with a companion cell which is as long as its associated element (figs.1 and 2).

During the development of the primary phloem elements, the precursors of the protophloem fibres outlying the periphery of the strands also gradually elongate until the petiole ceases to elongate. These fibres later develop secondary wall thickening and

lignification when secondary growth begins. Development of these fibres is more pronounced in the median strands and in the middle region of the petiole.

3.2.10.1 Secondary phloem

Initiation of secondary growth in the petiole of Salvadora is signalled by the differentiation of included phloem, differentiation of xylary fibres at certain loci and lignification of protophloem fibres (figs.17 - 19, Section V)

The normal secondary phloem is a narrow zone of tissue which occurs outside the cambium (figs. 3 - 5). It includes sieve elements, companion cells and phloem parenchyma. The included phloem forms a conspicuous feature of the petiole vasculature. Secondary phloem sieve elements are shorter than late formed metaphloem elements. Sieve plates are inclined (fig.6).

3.2.10.2 Development of the included phloem

The included phloem is a product of the vascular cambium produced by its abnormal activity. It is mentioned earlier that soon after secondary growth has commenced the cambium at certain loci forms towards inside some tangentially elongated groups of thin walled

derivatives instead of xylem (fig.21, Section V). After some time cambium resumes the normal activity so that the patches or islands of differentiating phloem cells become embedded in the xylem. Subsequently some of the central cells in the island differentiate into one or more groups of sieve tube elements, companion cells and phloem parenchyma. In some instances the complete differentiation of the derivatives may start even before they are actually embedded in the xylem so that at certain loci phloem may be seen on both sides of the cambium (fig.3). The included phloem islands appear more or less oval or circular in transections (fig.7). During the development of the included phloem islands of the adjacent strands may appear confluent with each other separated by interfascicular parenchyma.

Secondary growth first begins with the included phloem followed by the development of outer secondary phloem and secondary xylem. Usually only one large island of included phloem is noticed per strand but few small ones may also differentiate as secondary growth advances.

3.2.10.3 Comparison between secondary phloem and included phloem

Secondary phloem and included phloem are the products of the vascular cambium and both of them reveal

similar features except that in included phloem the elements are arranged in perfect radial rows (figs. 8 and 9). In the outer secondary phloem only the newly formed elements show radial seriation. Dimensional features of the early formed,late formed and secondary phloem sieve tube elements and companion cells are given in table VII.

3.2.10.4 Obliteration of sieve elements

Obliteration is first noticed in the median vascular strands at the basal region of the petiole at the fourth node. Early formed sieve elements develop during early elongation of the petiole, and within a short time, they become stretched and obliterated. Their position is indicated by the aggregated callose deposition. Late formed sieve elements remain functional comparatively for a long time. Some of them show signs of their senescence during the early stage of secondary growth. The radial extent of the secondary phloem shows only gradual increase during the life span of the leaf.

The process of obliteration is the same as that described in Crataeva. The obliterated sieve elements are noticed with heavy deposition of callose at the sieve plate region (fig.10).

3.2.10.5 Secondary phloem production in the mature petiole

Activity of the cambium and the production of secondary vascular elements are studied for a period of about 10 months. The differentiation of the secondary vascular elements is gradual throughout the life span of the leaf. The cambial zone is 2 to 4 cell layered throughout the growth period. Secondary xylem differentiation is more than that of the secondary phloem and included phloem (figs 12 - 14, 17).

3.2.11 Secondary phloem in the bark

As seen in transection the phloem cells of the axial system occur in radial series (fig.25, Section V). The secondary phloem includes sieve tube elements, companion cells, phloem parenchyma and sclereids. A large number of included phloem islands is present in a stem of about 2 cm diameter. Sieve elements are arranged in radial rows which differentiate from storied fusiform initials. Simple sieve plate is on the oblique end walls. P-protein is accumulated in the form of plug in the sieve elements mostly on one side. Companion cells vary in number and are arranged in a row on one side of the sieve element (fig.11).

Comparative feature of the secondary phloem in the petiole and stem is given in table VIII.

3.2.12 Metabolic activity of the phloem

Metabolic activity of the phloem and contiguous cells is investigated with thiazolyl blue. As in Crataeva, the cells which are metabolically active reduce the dye to give a blue colouration. In the vasculature, included phloem is more darkly stained than outer secondary phloem (fig.15). In the phloem, companion cells are more darkly stained than sieve elements indicating their high metabolism (fig.16).

Table VII : Dimensions of the primary phloem and secondary phloem sieve tube elements and companion cells in the petiole. Dimensions are in µm. Value in brackets shows the range.

		Length		Width	
		STE µm	CC µm	STE µm	CC µm
Primary Phloem	Early formed phloem	78.5 (61.23-97.34)	80.07 (59.66-95.7)	4.08 (3.14 - 4.17)	2.64 (1.57 - 3.14)
	Late formed phloem	72.22 (58.09-80.07)	72.22 (58.09-78.5)	5.79 (4.71 - 6.28)	2.64 (1.57 - 3.14)
Secondary Phloem	Outer Sec. phloem	67.51 (53.38-76.93)	64.37 (53.38-76.93)	5.81 (4.71 - 6.28)	2.75 (1.57 - 4.71)
	Included Phloem	65.94 (50.24-70.65)	62.80 (50.24-69.08)	5.99 (4.71 - 6.28)	2.66 (1.57 - 3.14)

Table VIII: Comparative features of the secondary phloem elements in the petiole and stem. (July collection)

	Petiole	Stem
Tissue types	Axial system	Axial system and ray system
Components of the Sec.phloem	Sieve tube elements, companion cells and phloem parenchyma	Sieve tube elements, companion cells, phloem parenchyma, ray parenchyma and sclereids
Sieve tube element		
Length µm	72.22	130.31
Width µm	5.81	12.56
End wall	Inclined	Inclined
Sieve plate	Simple	Simple
Diameter µm	6.28	15.70
Companion cell		
Length µm	69.08	45.53
Width µm	2.75	4.71
Number	Mostly one	2 - 4

Plate XXV

Figs. 1,3-5. Transections of the petiole. A portion enlarged.

Fig. 1. Vascular strand showing late formed metaphloem sieve elements. Early formed elements are mostly obliterated which occupy the inner periphery of the developing phloem fibres. (Arrows point to the obliterated sieve elements).

Fig. 2. Longitudinal view of the late formed metaphloem sieve elements (arrows point to the P-protein plugs; CC=companion cell).x 650.

Figs. 3 and 4. Recently formed secondary phloem elements. Radial seriation is not clear. (SP=Secondary phloem; INP = Included phloem). Fig.3. x 500.,Fig.4. x 300.

Fig. 5. Secondary phloem (SP) in a mature petiole. Radial seriation becomes more clear. x 150.

Fig. 6. A secondary phloem sieve tube element (arrow) with an associated companion cell (CC=Companion cell). x 750.

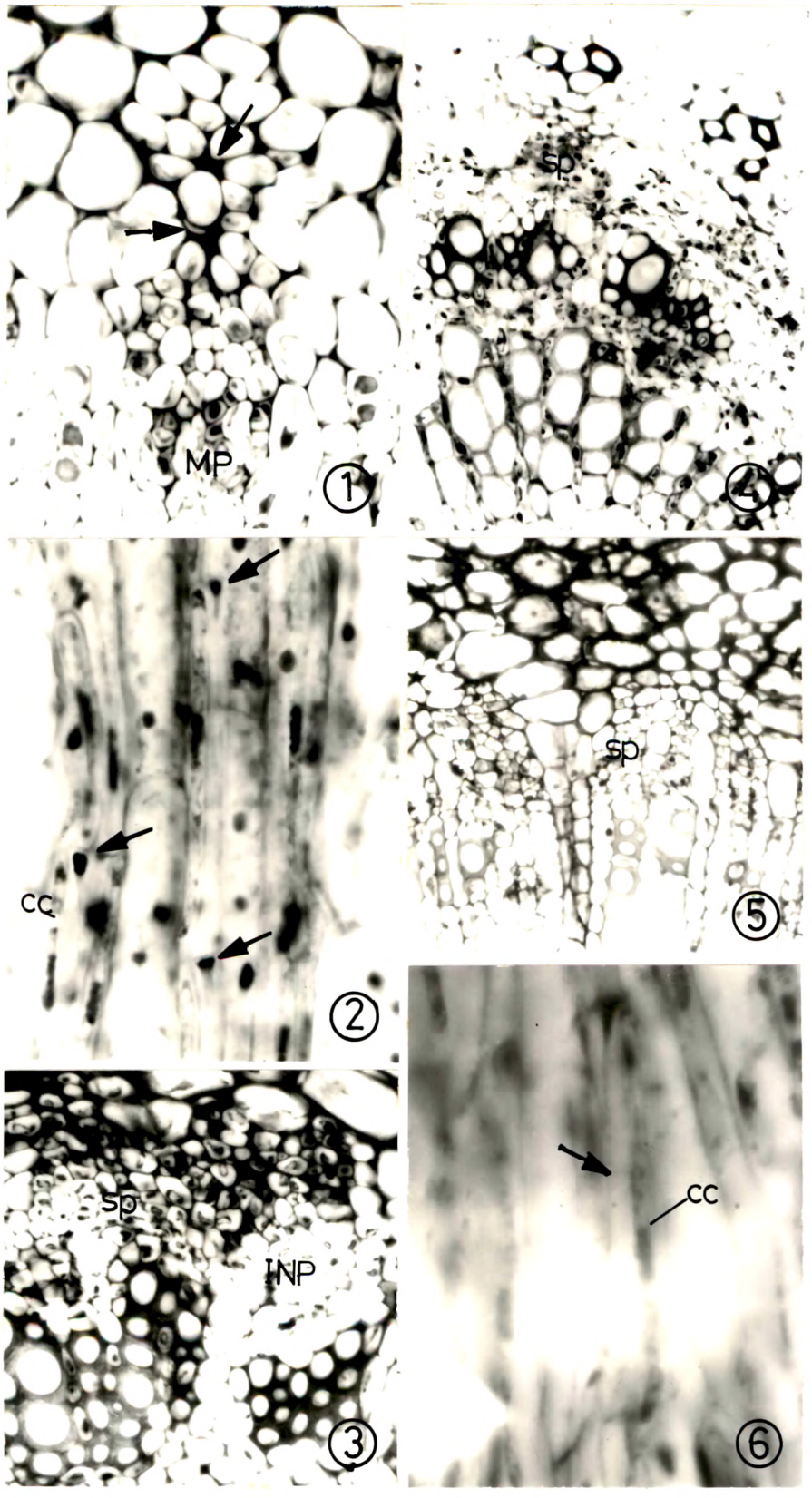

MP
1
sp
4
CC
2
sp
5
sp
INP
3
CC
6

Plate XXVI

Fig. 7. Transverse section of an included phloem island. Note the peripheral cambiform cells. x 750.

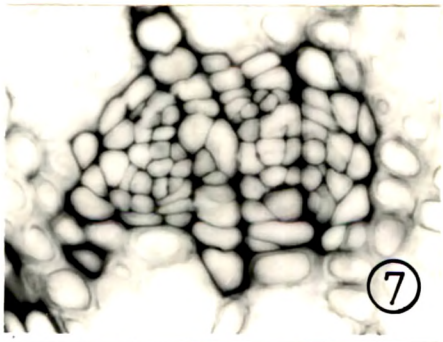
Fig. 8. Portion of the included phloem from a mature petiole. The phloem elements are arranged in radial rows. x 330.

Figs. 9 and 10. Longitudinal sections of the petiole.

Fig. 9. Included phloem. Arrows point to the companion cells. x 360.

Fig. 10. An obliterated sieve tube element with heavy deposition of callose on the sieve plate (arrows). x 370.

Fig. 11. Secondary phloem sieve tube element from the bark. x 313.



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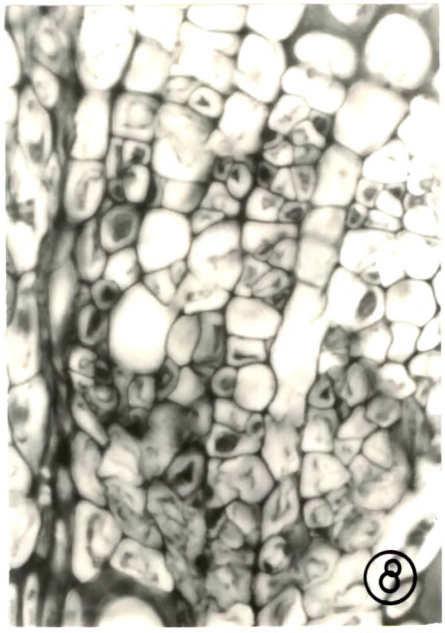

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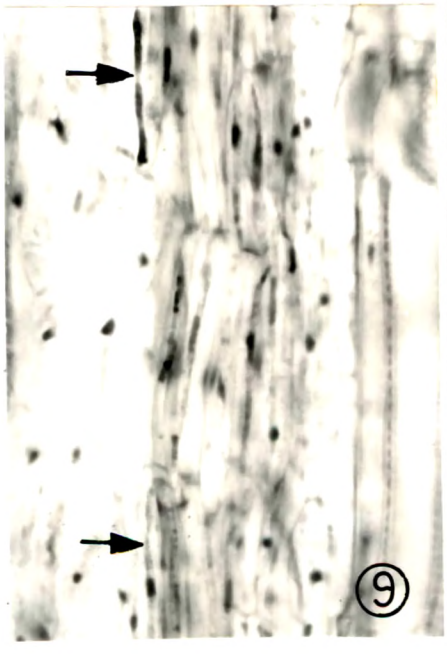

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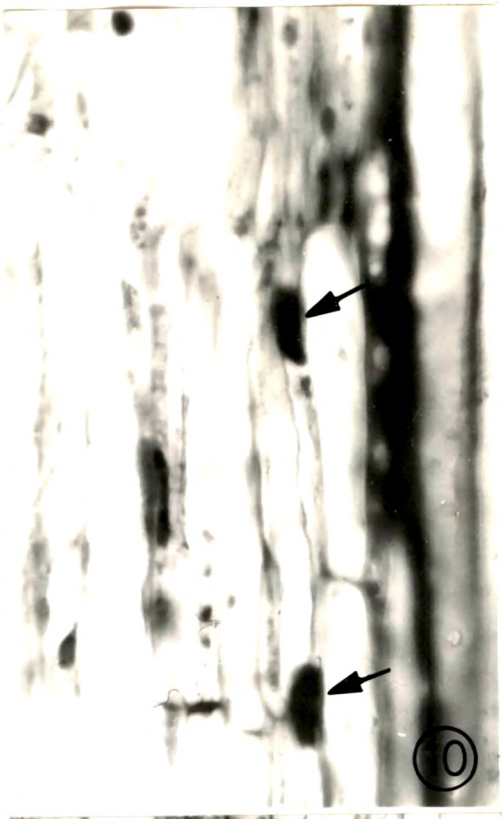

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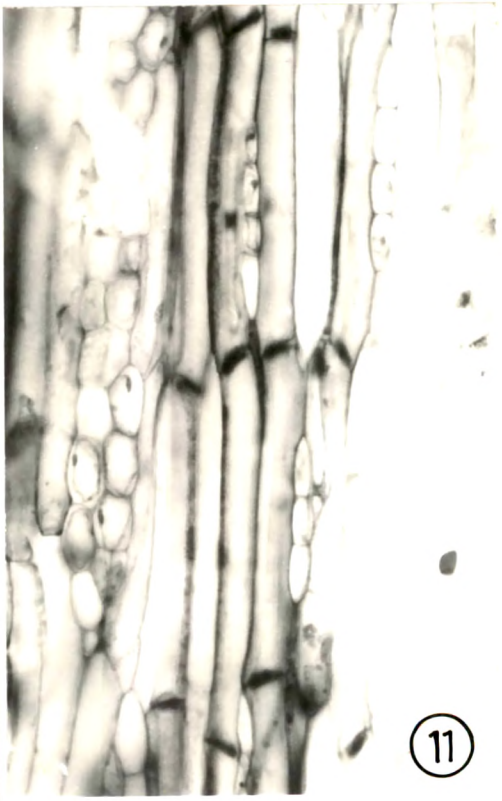
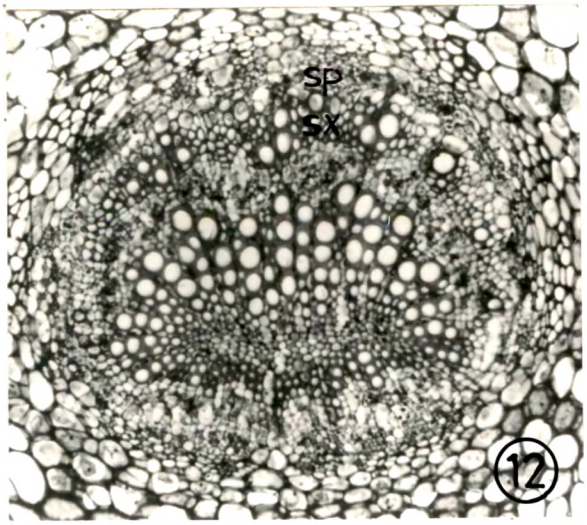


⑪

Plate XXVII

Figs. 12-14	Transection of the petiole showing the vasculature. All x 188.
Fig. 12.	Two month old petiole.
Fig. 13.	Five month old petiole.
Fig. 14.	Nine month old petiole. Note the radial diameter of the secondary phloem (SP) remains more or less the same whereas secondary xylem (SX) shows an increase.
Figs. 15 and 16.	Thiaxolyl blue localization to indicate metabolic activity of phloem cells.
Fig. 15.	Transection of the mature petiole. Included phloem shows more activity than outer secondary phloem (INP = Included phloem). x 230.
Fig. 16.	Longitudinal view of the vasculature. Sieve tube elements show activity (arrow). x 370.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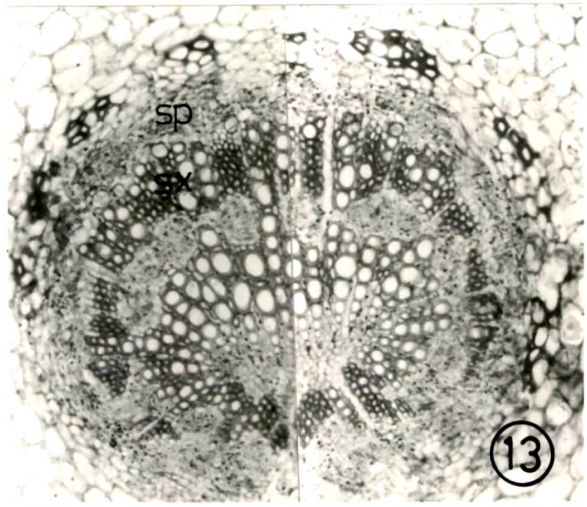
12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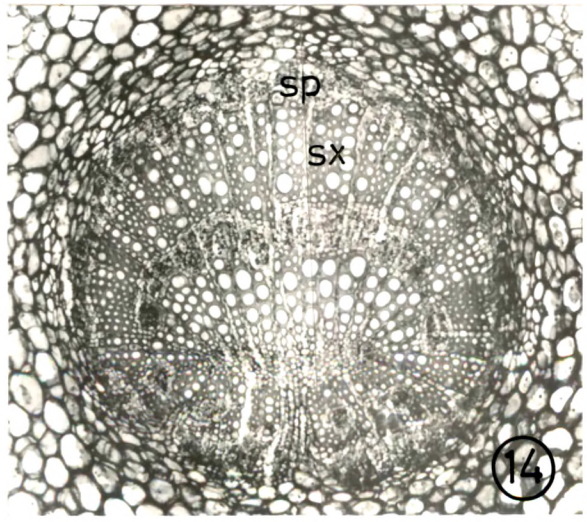
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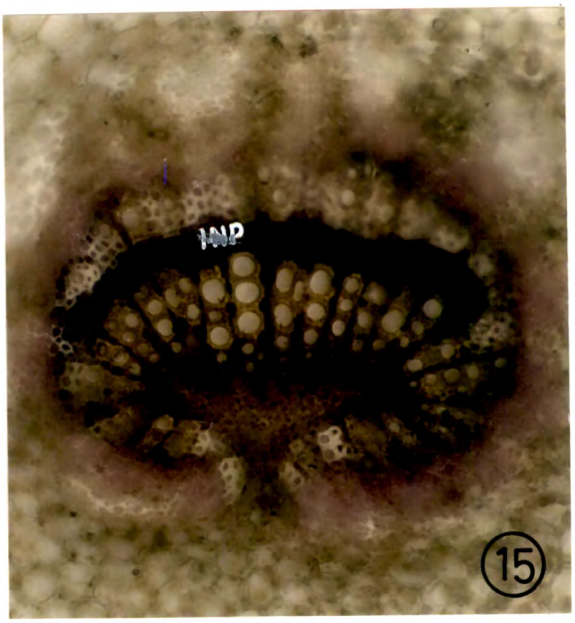
INP
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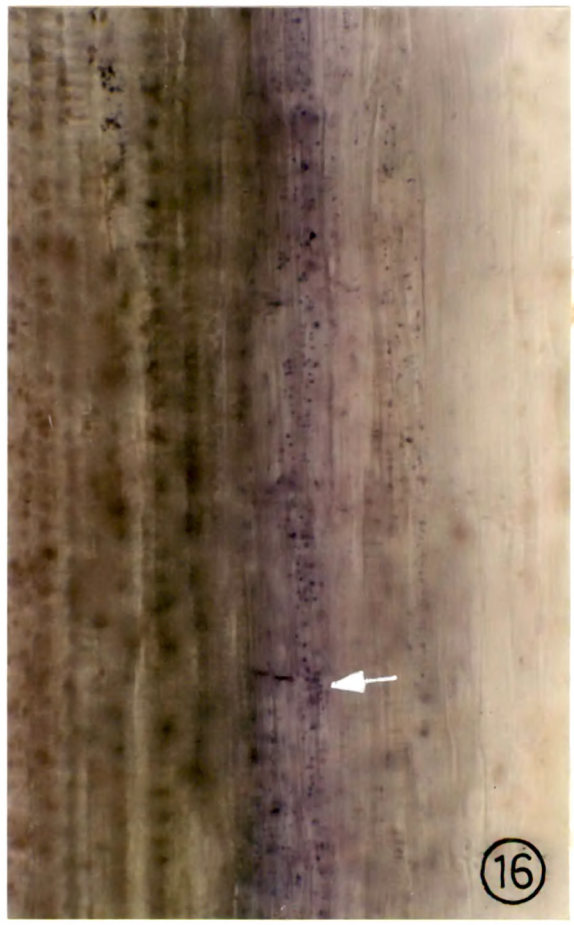
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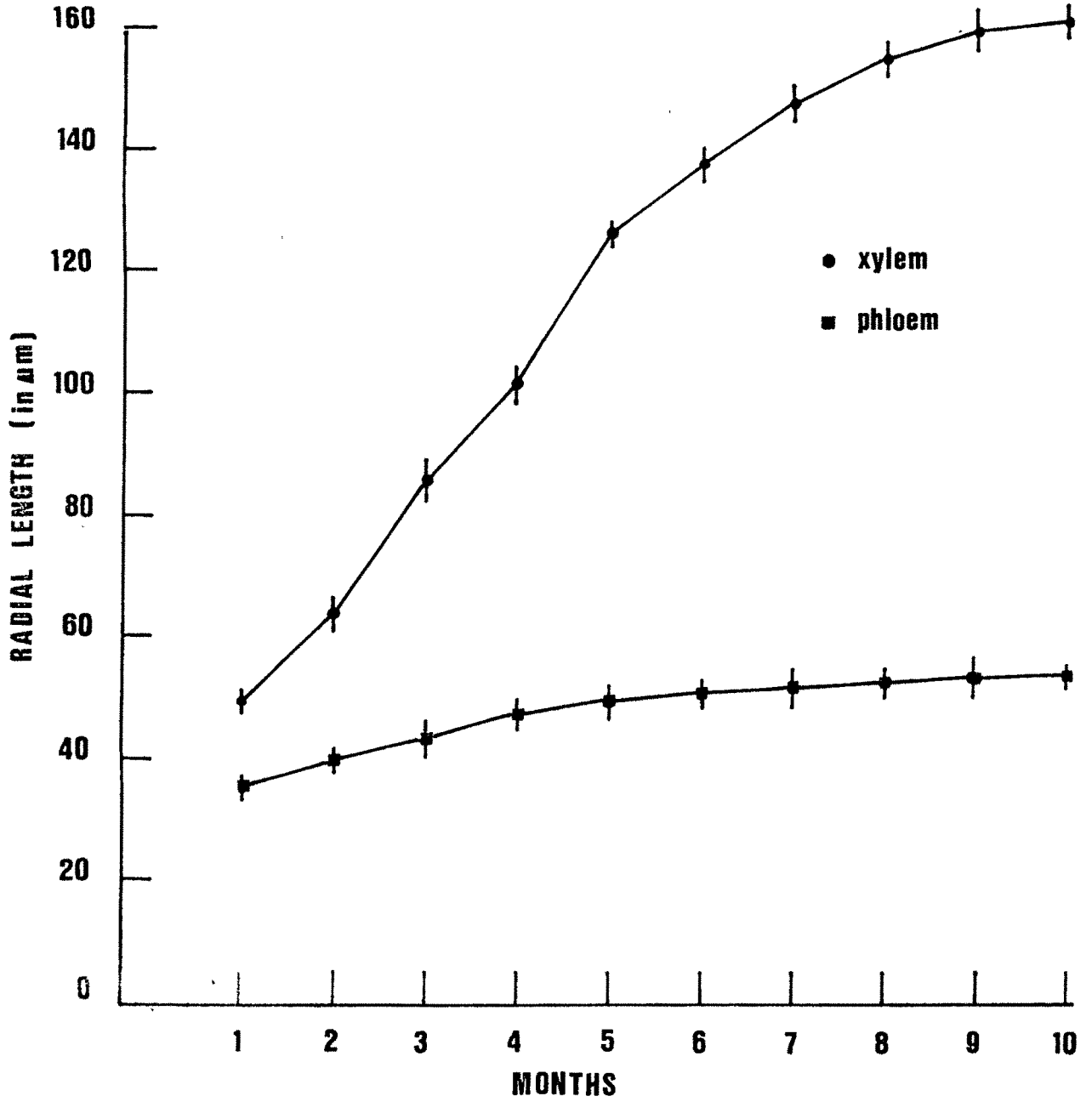
Figure 17. Radial length of phloem and xylem (in the median transverse sections) in 10 month old petiole over time.


160
140
120
100
80
60
40
20
0
RADIAL LENGTH (in µm)
xylem
phloem
1
2
3
4
5
6
7
8
9
10
MONTHS