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PRIMARY VASCULATURE DIFFERENTIATION IN *IPOMOEA SPS*

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ABSTRACT

The type of seed germination is epigeal. Phloem differentiates at lower levels than xylem and the root is tetrarch. A gradual inward shift in the pole of xylem differentiation results in transition from root to shoot structure. The root -hypocotyl- cotyledonary and epicotylar vasculatures are ontogenetically separate and establish connection below the cotyledons. The cotyledonary node is unilacunar two trace.

Keywords: *Seedling Anatomy*

INTRODUCTION

Earlier literature on seedling anatomy is confined to a study of the transition region. The present study aims at a correlated report of vascular differentiation, transition from root to shoot structure, connection between the root- hypocotyl-cotyledon and epicotyls vascular units and cotyledonary node.

MATERIALS AND METHODS

The seed of *Ipomoea palmata* and *Ipomoea pentaphylla* and *Ipomoea pulchella* were collected from Botanical garden of Dept. of Botany, Univ. of Raj. The seeds of three plant species were soaked overnight in water at room temperature before germinating them in petriplates lined with blotting paper. The radicular apices were dissected out from mature embryos. Root tips from growing seedlings at intervals of 24 hours within a week after radical emergence were also fixed in Formalin- Acetic acid-Alcohol (FAA) consisting of formalin, acetic acid and 70% ethanol in a proportion of 1:1:18 for about 48 hours and dehydrated through tertiary butyl alcohol (TBA) series and embedded in paraffin. Serial transverse sections of seedlings were taken at 10-12 μ m. and affixed the paraffin ribbons to slides. Houpt's adhesive was used for affixing solution. The dried slides were then stained with Tannic acid-Ferric chloride, saffranin and light green combination (Johansen, 1940). Cleared preparations of whole seedlings were studied. The vasculature in the seedling is traced from radicular to plumular end.

RESULTS AND DISCUSSION

Observations

The mature embryo shows procambial strands in the radical, plumule and cotyledons. The procambial cylinder surrounds an undifferentiated mass of cells in the axis. Germination is epigeal. During the first 24 hours of germination the radical and lower portion of hypocotyl elongate at a faster rate than the rest of the seedling. Elongation of the upper hypocotyl and the cotyledons become more noticeable during the second day.

Primary Tissue Development and Formation of Root Structure

From root to shoot tip of the four days old seedling measures about 15mm. At the root pole the differentiation of protophloem is followed by protoxylem. The protophloem initials are seen as four densely stained cell groups of phloem initials alternating to four xylem initials are developed (Figures 1A, 1B, 2A, 2B, 3A, 3B). The central part of the stele differentiates into the pith precursors which are broader than the procambial cells. Parenchyma cells in the pith and the cortex are pentagonal, hexagonal and octagonal to isodiametric in shape having intercellular spaces. A typical tetrarch root structure is established (Figures 1C, 2C, 3C, 3D). The number of xylem cells increases at higher levels due to differentiation of *in situ* meristematic cells in the pith region.

Transition from Root to Shoot Structure

The phloem groups start extending laterally at higher levels in all three species of *Ipomoea* and lateral extension of each metaxylem follows this (Figures 1D, 2D, 3D). This is followed by emergence of lateral

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roots from the pericycle in *Ipomoea pulchella* (Figure 3E). Metaxylem arms separate from the protoxylem groups at higher levels in all the three species and collateral condition was achieved where four collateral groups were formed each having a laterally extended phloem outside two metaxylem groups.

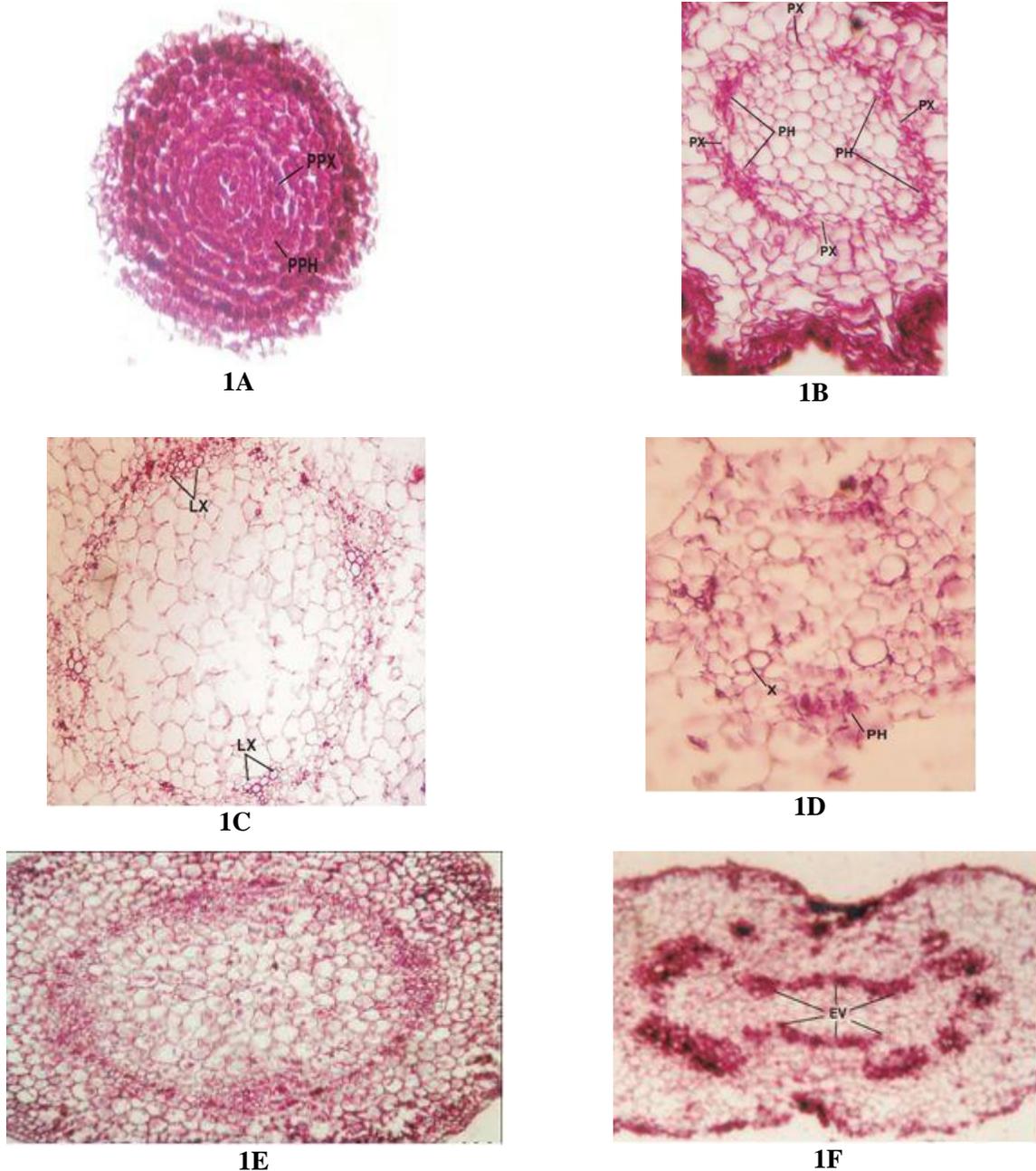


Figure 1: Serial transverse section of seedling of *Ipomoea palmata*

1A Differentiation of xylem and phloem X 400, **1B** Formation of xylem and phloem starts along with developed pith X400, **1C** Showing typical tetrarch root structure X400, **1D** Showing lateral extension of xylem arms X100, **1E** Separation of metaxylem arms along with bifurcation of the phloem groups and formation of eight collateral groups around the pith X100, **1F** Showing cotyledonary traces and epicotyls vasculature X100. PH phloem, PPH protophloem precursors, PPX protoxylem precursors, PX protoxylem, X xylem, LX lateral extension of xylem arms, EV epicotylar vasculature

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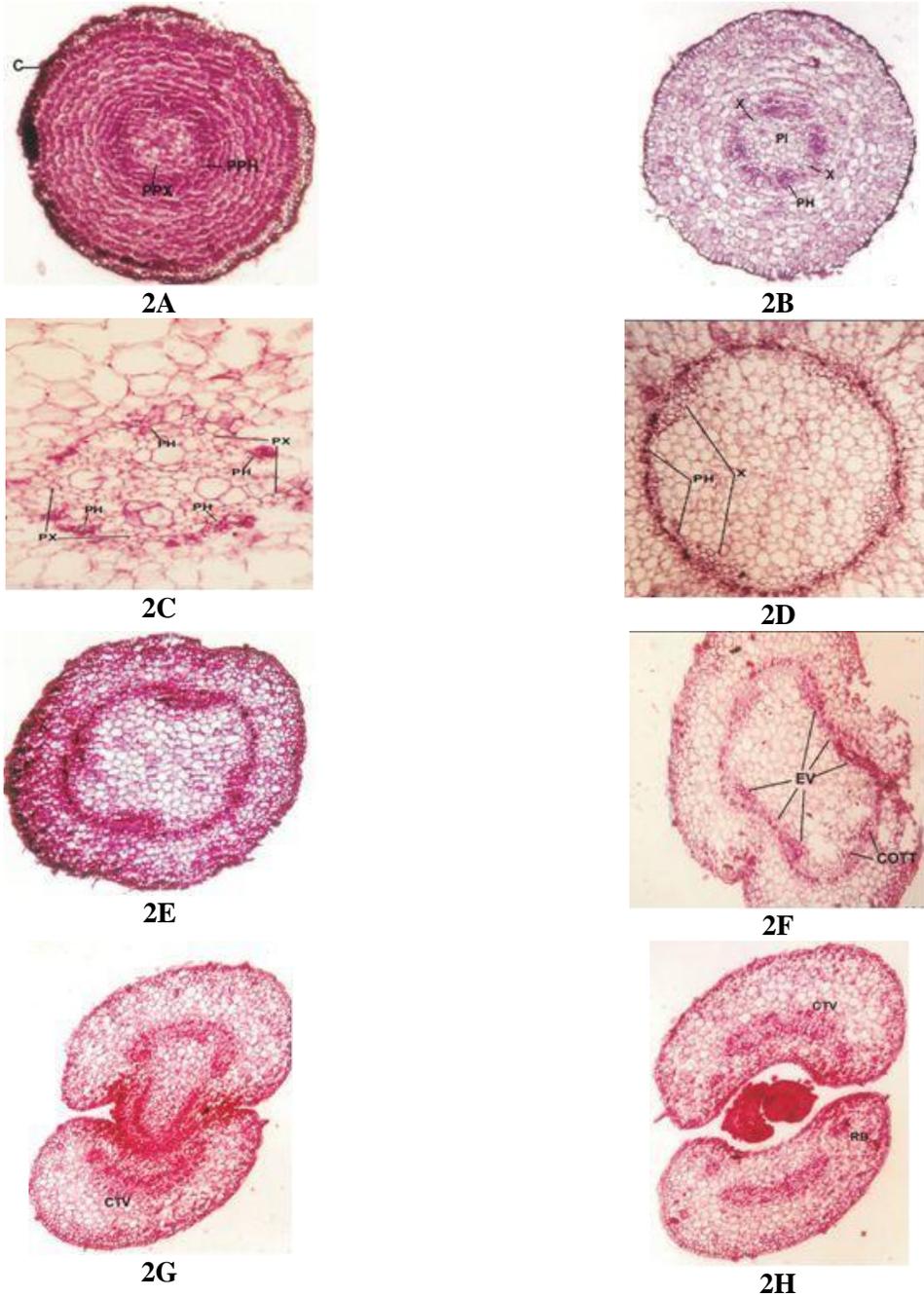


Figure 2: Serial transverse section of seedling of *Ipomoea pentaphylla*

2A Differentiation of phloem and xylem precursorsX400, **2B** Showing formation of phloem and xylem with developed pithX400, **2C** Typical tetrarch root structureX400,**2D** Formation of lateral extension of xylem and phloemX100, **2E** Separation of metaxylem arms along with bifurcation of the phloem groups and formation of eight collateral groups around the pith alternating with the protoxylem poleX100, **2F** Showing preparation of cotyledonary traces and differentiated epicotylar vasculature is seenX100,**2G**Fusion of the cotyledonary tracesX100,**2H** Showing division of cotyledonary trace vasculature and bundles supply to ridgesX100. PH phloem, PI pith,PPH protophloem precursors, PPX protoxylem precursors, PX protoxylem, X xylem, LXlateral extension of xylem arms ,CTV cotyledonary trace vasculature, RV ridge bundleEV epicotylar vasculature

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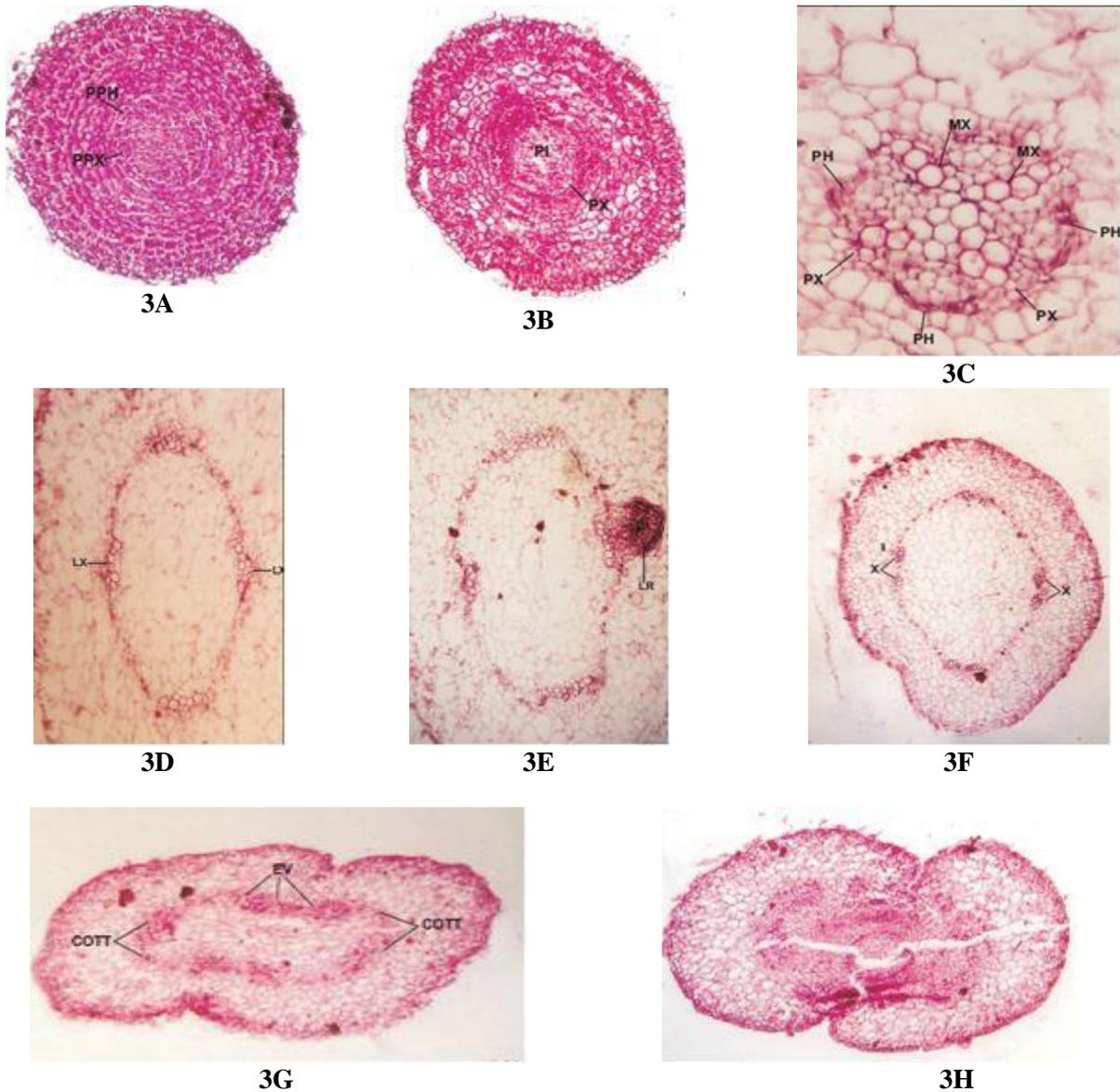


Figure 3: Serial transverse section of seedling of *Ipomoea pulchella*, 3A Differentiation of phloem and xylem precursors X400, 3B Formation starts of xylem and phloem tissue X400, 3C Typical tetrarch root structure X400, 3D Fully extended xylem and phloem arms forming an oval shape structure around pith X100, 3E Emergence of lateral root from pericycle region in lateral extended stage X100, 3F Separation of metaxylem arms along with bifurcation of the phloem groups and formation of eight collateral groups around the pith pole X100, 3G Showing epicotylar vasculature and cotyledonary trace X100, 3H Fusion of cotyledonary traces X 100, PH phloem, PI pith, PPH protophloem precursors, PPX protoxylem precursors, PX protoxylem, X xylem, LX lateral extension of xylem arms, COTT cotyledonary trace, EV epicotylar vasculature, LR lateral roots

The metaxylem arms was observed along with bifurcation of the phloem groups which resulted in formation of eight collateral vascular groups, each having only one xylem group with a phloem arc outside (Figures 1E & 2D).

In between the two such collateral groups and on the outer flanks conjunctive tissue joins them and a continuous ring of vasculature is formed.

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This conjunctive tissue becomes sclerenchymatous later near the cotyledonary plate; there is a separation of the two primary xylem units formed due to bifurcation of the original diarch xylem plates. One of this unit becomes the vascular trace of one of the cotyledons and the second unit, becomes vascular trace of the other cotyledons.

Vascularization of the Cotyledons

The hypocotylar vasculature acquires almost elliptical shape with four collateral vascular groups, each containing one or two arms of xylem separated by parenchymatous cells and one phloem arc outside.

Complete obliteration of the protoxylem groups takes place at higher levels in all the three species. The eight collateral bundles are drawn in the form of two arcs and bifurcates (Figures 1E, 2D, 3F). Two groups in the cotyledonary plane separate out as cotyledonary traces. The remaining four groups in the intercotyledonary plane fuse to form two composite bundles, these two bundles are placed opposite to each other. Basipetally differentiating epicotylar procambium is observed between each pair of bundles in the intercotyledonary plane (Figures 1F, 2E, 3G).

Each cotyledon is supplied by a pair of one bundle, one from each arc, thus the cotyledonary nodal condition is unilacunar two trace. In all species the two cotyledonary traces remain close to each other at the base of cotyledons and fuse at higher levels and then split in the cotyledonary lamina and supply it. Differentiation of tissue takes place from the epicotyl which closes the cotyledonary gaps after axillary bud vasculature separates out from the axis (Figures 1F, 2F, 2G, 3H).

Discussion

There are only a few reports on early differentiation of primary vascular tissue in angiosperms. Vascularisation of the seedling is of particular interest because it is the first representation of the vascular coordination between root and shoot for shadowed in the procambial system of the embryo. Investigations, which help to build an overall picture of this coordination, are of great value (Banerji, 1961; Kavathekar and Pillai, 1977; Pillai and Goyal, 1979). The present reports also agree with this. Majority of available reports suggest that the primary phloem is the first to differentiate than the xylem. Sharma and Sharma (1985) reported the differentiation of protophloem initials closer to the meristem in *Mimosa*. The three species studied here, showed that the protophloem initials are seen first as four denser staining recognizable groups of cells near the inner boundary of the procambial cylinder whereas the protoxylem initials differentiate near the outer boundary of the procambial cylinder and at alternate positions to the protophloem groups. Here the protoxylem maintains its original position until the level just below the cotyledonary node. The initial centripetal differentiation of the metaxylem is followed by lateral extensions and differentiation of parenchyma cells in between the two metaxylem arms. This pattern of change over from radial to collateral condition formation is followed in all the species. The earlier workers like Eames and Mc Daniels (1947), Banerji (1961) and Govil (1973) however, reported splitting and rotation of xylem through 180° during transition from root to shoot structure. But the data reported here are in support of Bonnier's (1900 a,b) supported by Kavathekar and Pillai (1977) and Sharma *et al.*, (1984) conclusions that the transition from radial to collateral condition takes place through gradual inward shift of pole of xylem differentiations as we ascend along the axis. Deshpande and Kasat (1966), Govil (1973), Pillai and Kumar (1979) reported connection between the root - hypocotyl and epicotyl vasculatures through the first foliar node. The species studied here the root - hypocotyl vascular unit completely enters into the cotyledons and the first foliar leaves receive epicotylar vasculature. The downwardly differentiating epicotylar vasculature is seen at the time of departure of cotyledonary traces. The root – hypocotyls and epicotyl vascular units are separate and tend to support the Thoday's (1939) suggestion of double origin of vasculature. The cotyledonary node in all three species showed a unilacunar double trace condition. This condition has also been reported in different families of angiosperms. Literature studied revealed that there are different types of cotyledonary nodes in angiosperms (Canright, 1955; Eames, 1961; Carlquist, 1961; Sharma, 1981). Marsden and Bailey (1955) discussed the evolutionary tendencies and considered the unilacunar double trace nodal condition to be primitive. Bailey (1956) suggested that many plants have unilacunar nodes and there is no record where a trilacunar three-trace condition of the cotyledonary node is succeeded by a unilacunar one trace. This was in contrast to the Benzing's (1967a,b) observations that the primitive node

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in angiosperms is one trace unilacunar or trilacunar. The prevalence of unilacunar double trace condition at the cotyledonary nodes in widely separated taxa like *Annoa*, *Asclepias*, *Calotropis*, *Delphinium*, *Papaver*, *Polyalthia* and *Sesamum* (Kavathekar *et al.*, 1979), some Crucifereae (Goyal, 1981; Babber, 1981), some Mimosideae (Sharma, 1981), some tree legumes (Dubey, 1986) some *Cassia* species (Pathak, 2001) as also in the species reported here tend to support the contention that the unilacunar double trace condition might be the ancient type of nodal condition.

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