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# Studies on the Arrangement and Structural Anatomy of Xylem Tissue in the Transition Region of Three Legume Species

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## Abstract

The arrangement of vascular tissues differs in the root and shoots in angiosperms. The root has a solid triarch or tetrarch exarch xylem whereas the stems possess endarch xylem made of individual vascular bundles arranged in a ring form. A continuous flow of water and minerals is not disrupted in spite of this difference in the arrangement of xylem tissue in the two organs. This happens because of a transition region extending from the upper portion of the root, ending in either the hypocotyl or epicotyl where a gradual alteration in the arrangement of the xylem tissue occurs. The aim of the study was to investigate the extent of transition region and the structural anatomy of xylem in the transition region of three tropical legume species *viz. Vigna radiata*, *V. unguiculata* (red variety) and *Cicer arietinum*. Sequential transverse sections from the base of the root, hypocotyl and epicotyl were obtained from five day old seedlings of the three selected legumes, stained with safranin and viewed under the light microscope. Sections were digitally photographed. In all the three species studied, the transition region commenced a few millimeters below the soil surface. In *Vigna radiata* and *Vigna unguiculata* it ended in the hypocotyl region above the soil surface whereas in *Cicer arietinum* it ended in the epicotyl region. The beginning of transition region showed appearance of a pith in the center of the xylem core. Gradual widening of pith resulted in separation of xylem tissue. This was followed by reorientation of exarch to endarch xylem. A variation in the structural anatomy of xylem among the three species was also observed in the length of transition region and structural anatomy of xylem.
**Keywords:** *Vigna radiata*, *Vigna unguiculata*, *Cicer arietinum*, xylem, transition region.


## INTRODUCTION

In angiosperms, the arrangement and orientation of differentiation of the primary vascular tissues differ in the root and stem. In roots, the development of the protoxylem is exarch whereas in the stem it is endarch. In dicot roots, the xylem tissue is a solid triarch or tetrarch. In some dicot stems, the primary vascular cylinder is made up of individual vascular bundles arranged in a ring form. Despite these differences in arrangement of vascular tissues in root and stem, the transport of water and minerals is not disrupted and a continuous flow is maintained between the two organs. What makes this possible is the alteration in arrangement of vascular tissue in the stem and root. The exarch protostele of xylem in the roots with independent phloem strands must be merged smoothly with the endarch collateral bundles of the shoot. The zone of the plant axis where the arrangement of vascular systems changes from root to stem structure is known as "transition region" [1-4]. There is variation in the structure and positioning of this region amongst species [5].

The region remains in the hypocotyl entirely as in *Melilotus alba* [6], *Aabidopsis thaliana* [7] *Rhynchosia edulis, Rh. senna, Galactia marginalis,* and *G. latisiliqua* and *Clitoria cordobensis* [8] or extend to the lower nodes of the stem in the epicotyl as in *Pisum sativum* [9]. The length of the region partially depends on the mode of hypocotyl elongation [10, 11].

Many workers have conducted studies on the transition region of different legume species [9, 11, 8, 12-14]. This study describes in details the extent of transition region and the structural anatomy of xylem in the transition region of three tropical legume species *viz. Vigna radiata*, *V. unguiculata* (red variety) and *Cicer arietinum*.

## METHODOLOGY

The three species selected for study, *Vigna radiata* and *Vigna unguiculata* and *Cicer arietinum* belong to the Fabaceae family. They are all herbaceous plants utilized as pulses.

Seeds were obtained from grocery store, were sown in washed sea sand (for easy harvesting) in thermo cool boxes and placed in the corridor. Seeds and seedlings were watered on alternated days. Five day old seedlings were harvested and free-hand sections were prepared for the anatomical studies. Thin sections were selected and stained with safranin. The sections were examined under the light microscope (Leica Microsystems (Schweiz) AG, Wetzlar, Germany) at 10x magnification and pictures were taken of the stele with a digital camera (Nikon Coolpix L23, 10.1 megapixels, 5x zoom, Nikon Corp., China).

## RESULTS

*Vigna radiata* has epigieal development. Transverse section of the root at 7 mm below the soil surface shows exarch, radial and tetrarch protostele with four protoxylem arms and a large metaxylem region in the center. The protoxylem is a file of 3-6 single cells and the central metaxylem is a group of 35-40 cells forming a central circular region (fig. 1a). As one moves towards the base of the stem, the central part of solid metaxylem cells is seen replaced by parenchyma cells which indicates the commencement of pith formation (fig. 1b). The pith region enlarges as there is a progression further towards the stem base, eventually splitting xylem tissue into four clusters. As the pith increases in diameter simultaneously, the radial width of the xylem clusters becomes narrower (fig. 1c – fig. 1f). Further movement towards the stem base shows progressive widening in pith diameter leading to wider gaps between the xylem which results in formation of four separate xylem clusters (fig. 1g – 1i). The metaxylem in these clusters is oriented laterally and the protoxylem faces the pericycle. This anatomical arrangement marks the end of the root region.

Transverse section at the base of the stem shows almost triangular xylem cluster with protoxylem at the apex facing the pericycle and metaxylem towards the center (fig. 1j). The metaxylem shows a gradual lateral divergence from the protoxylem ultimately forming almost linear cluster (fig. 1k – 1l). There is an inversion of the xylem cluster followed by the division of each cluster into two, orienting the protoxylem and metaxylem linearly (fig. 1m – 1o). The protoxylem is median in the two halves of the cluster. Further along the stem 9 mm above the stem base, xylem cluster is seen increasing in number and protoxylem gets oriented towards the center of the axis and metaxylem towards the outer region (fig. 1p – 1r). This marks the end of the transition region.

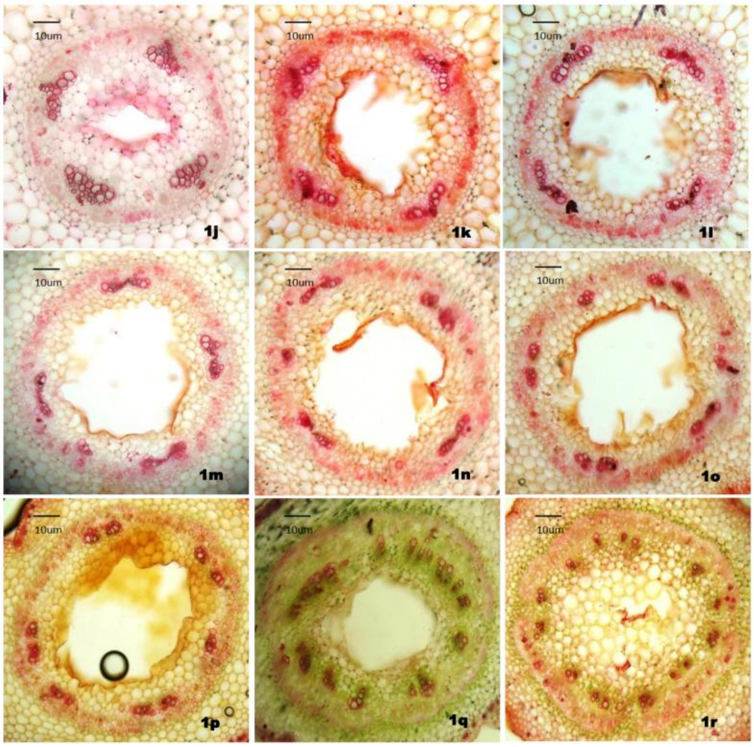
*Vigna unguiculata* has epigeal development. Transverse section of the root at 9 mm below the soil surface shows exarch, radial and tetrarch protostele with metaxylem in the center and four relatively longer multicellular radiating arms of protoxylem. The protoxylem arm is made up of 12-15 small sized cells and central metaxylem comprises of 16-18 relatively larger cells forming almost circular central core (fig. 2a). The transition zone starts in the region of the root approximately 8 mm below the soil surface. The parenchyma cells starts appearing in the central metaxylem zone, indicating the initiation of pith (fig. 2b). Further widening of the pith results in separation of the xylary tissue into four clusters (fig 2c – 2h). The widening of the pith is simultaneously accompanied by further separation and reduction in size of the four xylary clusters. The orientation of the xylem tissue is metaxylem towards the pith and protoxylem facing the pericycle.

Transverse section at the base of the stem reveals further division of each xylem cluster into two segments and their gradual linearization with the metaxylem and protoxylem maintain their same positions (fig. 2i). Above the stem base, there is further linearization of xylem segments aligning the metaxylem and protoxylem in the same plane with a periclinal orientation. The protoxylem is positioned at the ends of the segments facing each other (fig. 2j – 2k). The divided segments of each xylem cluster then moves further apart and there is gradual inversion of protoxylem towards the pith and the metaxylem towards the pericycle (2l – 2q). 11mm upwards from the base of the stem, the xylem is in form of separate radially oriented strands with protoxylem facing the pith and metaxylem facing the cortex (fig. 2r). This marks the end of transition zone.

*Cicer aerietinum* exhibits hypogeal type of germination. The transverse section of root at 9mm below the soil surface exhibited exarch, radial and tetrarch protostele with metaxylem towards the center and protoxylem at the apices forming four radiating multicellular arms (fig. 3a). The arms of protoxylem consists of 15-20 small sized cells radiating from the central 21-25 larger metaxylem cells forming almost rectangular shaped core. Further advancement towards the stem base shows appearance of parenchyma cells in the center of the metaxylem area which indicates the beginning of pith (fig. 3b – fig 3d). As the pith area enlarges, the metaxylem cells begin to form almost a ring around the expanding pith periphery and there is a reduction in the length of protoxylem arms (fig. 3e). At the base of the stem, there is further expansion of the pith with the metaxylem forming almost continuous ring around the pith and there is further reduction in protoxylem arm length (fig. 3f).

In the hypocotyl area, gaps appear in the metaxylem ring dividing it into segments (fig. 3g – 3h). At the cotyledonary node, the space between the xylem segments enlarges separating the segments. The protoxylem becomes reoriented to face the pith and metaxylem is in linear orientation periclinally to the pericycle (fig. 3i). The pith continues to expand in the epicotyl region, there is further splitting of xylem segments and the xylem segments assume almost 'v' shape with protoxylem towards the pith (fig 3j – 3m). The separated xylem segments moves further apart. At 1.4 cm above the soil surface, the xylem is seen arranged in a ring of distinct units indicating the initiation of discrete vascular bundles and end of transition zone (fig. 3n).


**Fig-1: Transverse sections showing structure of xylem tissue in the root and transition region of *Vigna radiata***
**1a: Transverse section (T.S.) of root with solid tetrarch xylem**
**1b: T.S. at 6 mm below the soil surface showing development of parenchyma cells in the central core of metaxylem indicating initiation of pith in the transition region**
**1c-1f: T.S. showing further expansion in pith diameter leading to splitting of xylem into clusters with eventual reduction in its radial width**
**1g-1i: T.S. showing wider separation of xylem clusters by expanding pith and reduction in protoxylem arm length (stages 1b – 1i: Occur below the soil surface above the root proper)**




**1j: T.S. through the stem base with four xylem clusters**
**1k-1l: T.S. above the stem base revealing gradual linearization of xylem clusters**
**1m-1o: T.S. through the hypocotyl showing splitting of linear xylem cluster with each segment consisting of protoxylem and metaxylem**
**1p-1q: T.S. showing inversion and further separation of xylem segments leading to protoxylem facing pith and metaxylem towards pericycle**
**1r: T.S. through hypocotyl at 9 mm above the soil surface. End of transition region**

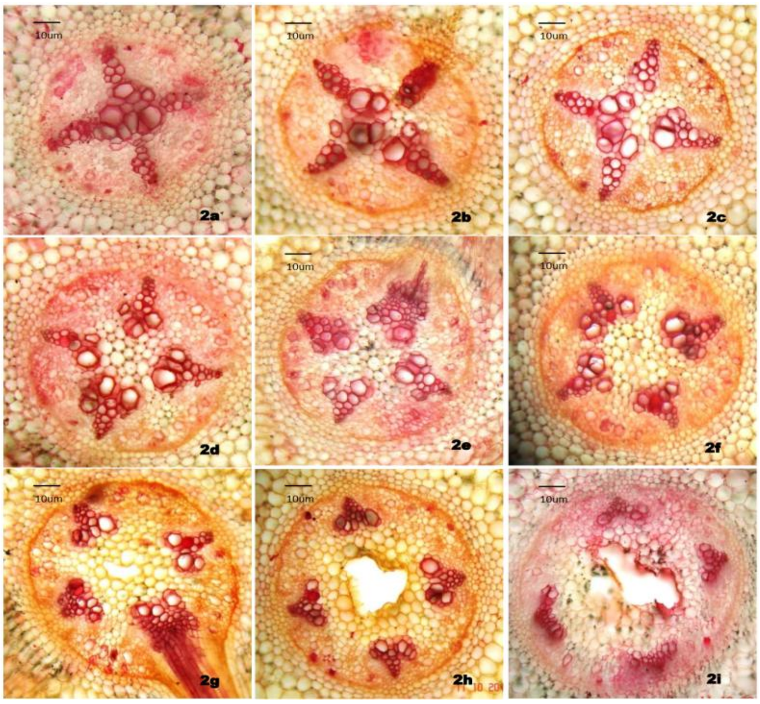
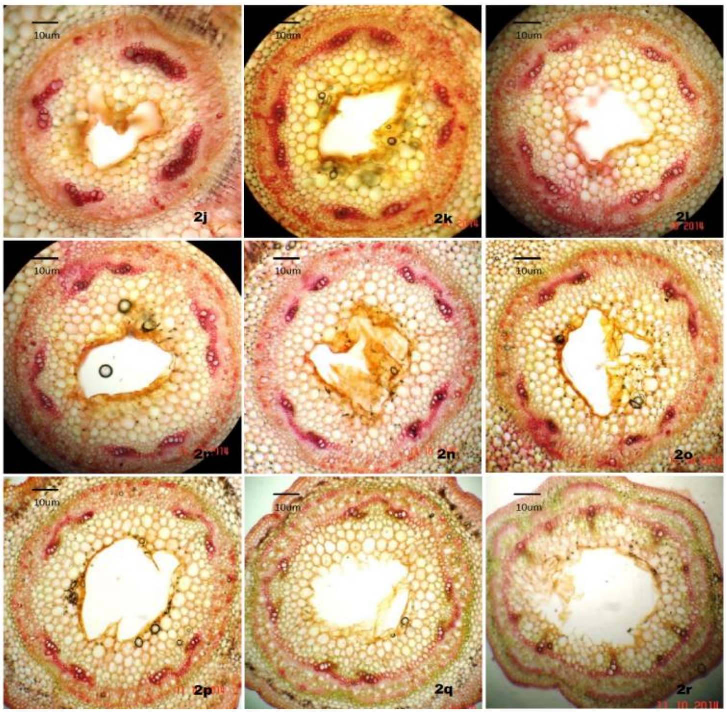



**Fig-2: Transverse sections showing structure of xylem tissue in the root and transition region of *Vigna unguiculata***
**2a: Transverse section (T.S.) of root with solid tetrarch xylem**
**2b: T.S. at 8 mm below the soil surface showing development of parenchyma cells in the central core of metaxylem indicating initiation of pith in the transition region**
**2c-2h: T.S. showing further expansion in pith diameter leading to splitting of xylem into clusters with eventual reduction in its radial width**
**2i: T.S. through the stem base showing gradual linearization of xylem clusters**



**2j-2k: T.S. through the hypocotyl region above the soil surface showing eventual splitting of each xylem cluster into two segments each with protoxylem and metaxylem**

**2l-2q: T.S. through the hypocotyl revealing gradual inversion of each xylem segments orienting protoxylem towards the centre and metaxylem facing pericycle**

**2r: T.S. through hypocotyl at 11 mm above the soil surface with showing discrete xylem segments. End of transition region**

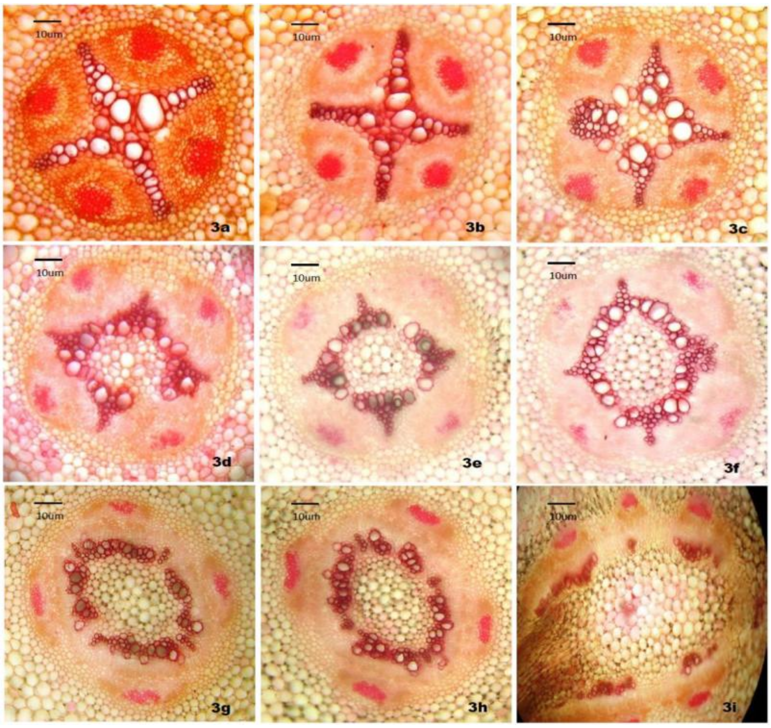


**Fig-3: Transverse sections showing structure of xylem tissue in the root and transition region of *Cicer arietinum***

**3a: Transverse section (T.S.) of root with solid tetrarch xylem**

**3b-3d: T.S. from 8 mm below the soil surface showing development of parenchyma cells in the central core of metaxylem indicating initiation of pith in the transition region**

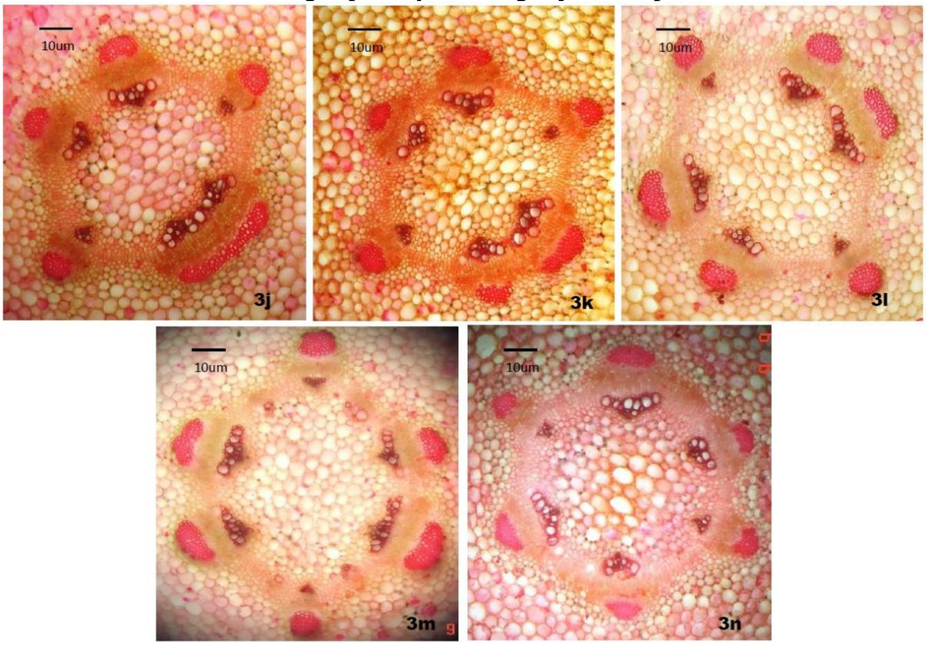
**3e: T.S. showing further expansion in pith diameter**

**3f: T.S. through the stem base showing metaxylem forming a ring around the pith**

**3g-3h: T.S. through the hypocotyl region above the soil surface revealing gaps in the metaxylem ring**

**3i: T.S. passing through the cotyledonary node showing segmentation of metaxylem and inversion of the xylem segment leading to protoxylem being adjacent to pith**




**3j-3n: Transverse section (T.S.) through the epicotyl region showing well developed pith with discrete xylem segments in a ring around the pith. The xylem segments assume 'v' shape with protoxylem facing the pith (1.4 cm above the soil surface). End of the transition region**

# DISCUSSION

The root-stem transition of an angiosperm is the portion of the axis where the arrangement of the vascular tissue of root changes into that of shoot. This leads to a change of a solid exarch, radial xylem with two or more radiating arms to discrete endarch xylem arranged in discontinuous ring surrounding pith. The transition zone has been studied in a number of species within the Fabaceae family [8, 12, 13]. The present study also looked at transition region in three species of Fabaceae.

In all the three legume species studied, the typical pattern of xylem in the transition zone was noted. There were differences in the level of commencement and end of the transition zone. The transition region and the arrangement of the xylary element differed in the three species.

Basconsuelo *et al.*, [8] indicated that according to Hayward [15] in the species of tribe Vicieae, the transition zone does not end in the hypocotyl but continue in the lower internodes of the stem (epicotyl region). Similar results were noted in the *Cicer aertinium* of tribe Ciceraeae whilst in the *Vigna radiata* and *Vigna unguiculata*, the transition zone ended in the hypocotyl similar to *Glycine max* [13] and species in the tribe Phaseoleae [8]. According to Basconsuelo *et al.*, [8], different species in the tribe Phaseoleae, exhibits similar arrangement of the xylem vessels in transition zone.

In the present study, the transition region in *Vigna radiata* commenced 7 mm below the soil surface and extended till 9 mm in the hypocotyl above the soil surface. El-Shaarawi *et al.*, [12] also reported that the transition region in *Vigna radiata* started 1 cm (10 mm) below the soil surface and continued in the hypocotyl region which is approximately similar to the results obtained in the present study.

The xylem anatomy of the three species studied showed variations. Transverse section of the root in all three species indicates that the xylem has a tetrarch arrangement (figs. 1a, 1b & 1c). However there are slight structural differences in the organization of the xylem tissue. In *Vigna radiata*, the arms of the tetrarch xylem are short consisting of a single column of cells (fig. 1a). The central core of the stele (metaxylem elements) is wider and near isodiametric. On the other hand, the tetrarch xylem of red variety of *Vigna unguiculata* has longer and multicellular arms with a smaller central core of metaxylem (fig. 1b). In *Cicer arietinum*, the arms of tetrarch xylem are long, multicellular and relatively wider (fig. 1c) than in the other two species. The middle core of tetrarch stele (metaxylem) is relatively narrow in diameter compared to *Vigna radiata* and square in shape.

In the *Cicer arietinum*, the xylem in the transition zone at the base of the stem is a continuous rectangular ring enclosing the pith in the center (fig. 3f). In the *Vigna radiata* and *V. unguiculata* however, the xylem in the transition zone at the base of the stem is broken into four discreet units separated by the pith (fig. 1g and 2f).

Many studies have revealed that the parenchymatous tissue of the pith are involved in effecting the changes between vascular tissues of shoot and root. The parenchyma of the pith in the transition zone changes the xylem structure from a solid tetrarch in a root to discrete units in the stem through segmentation and separation of solid xylem by its expansion. This results in a shifting of the structure from a central core of vascular tissue in the root to discrete units of vascular bundles inn the stem. This is attained by the formation of pith at the beginning of transition, repositioning of metaxylem & protoxylem and increase in the distance between the segments of xylem [16-18, 7, 8, 12, 13]. The present study also revealed the same anatomical arrangement and structure of xylem in the transition region that were noted in the above researches.

This study adds further knowledge on the anatomical structure, pattern of xylem arrangement and the mode of change in orientation of the protoxylem in the transition region of three members of Fabaceae.

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