# STEM ANATOMY AND DEVELOPMENT OF INTRAXYLARY PHLOEM IN *VALLARIS SOLANACEA* (ROTH) KUNTZE (APOCYNACEAE)

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A single ring of vascular cambium increases the stem thickness of *Vallaris solanacea* (Roth) Kuntze (Apocynaceae). During the ontogeny, procambium becomes distinct in the third visible internode and complete ring of the cambium is observed in fifth internode. Discrete strands of intraxylary phloem develop prior to protophloem in the fourth visible internode while additional intraxylary phloem derivatives are added subsequently from the adjacent parenchyma cells. Earlier formed phloem became non-conducting and accumulated centripetally due its obliteration. In fully grown thick stems, cambial action occurs external to the intraxylary phloem and differentiation of secondary xylem and phloem takes place in centrifugal and centripetal direction respectively. Diffuse porous secondary xylem with distinct growth rings consisted vessels, tracheids, fibres, axial and ray parenchyma cells. Dimorphic vessels showed drastic difference in their lumen diameter. The narrow vessels were thick walled and similar to tracheary elements with small and obliquely placed perforation plate on sub-terminal end. Vessels with wide diameter are mostly solitary while smaller vessels either appear in clusters or arranged in radial or diagonal multiples. Xylem rays are uni- to biseriate while multiseriate rays observed occasionally. Presence of radially arranged non-articulated laticifers in thick walled rays is frequent in all the samples investigated.

Keywords : climbing habit, internal phloem, non-articulated laticifers, fibriform vessels, *Vallaris*

In majority of dicots, phloem occurs on the outer side of the cambium but it may also occur embedded within secondary xylem or on the periphery of pith. Phloem strand that are embedded within secondary xylem that are originating from a single ring of cambium are called as interxylary phloem (Carlquist 2013). Sieve elements that differentiate between the pith and the proto-xylem are termed internal or intraxylary phloem. Occurrence of internal phloem was reported for the first time by Hartig (1854) in Cucurbitaceae and subsequently by Veseq (1875) in the Solanaceae and the Apocynaceae. Thereafter, its presence is documented in several species (Hanstein 1864, Scott and Brebner 1889, Solereder 1908, Esau 1938, 1969, Mikesell and Schroeder 1984, Patil *et al.* 2009, 2014, Zozimo *et al.* 2011). Available literature indicates that occurrence of intraxylary phloem is reported in 30 families of dicots (Metcalfe and Chalk 1983). Though, it has been reported in 30 families, yet it forms small portion of dicots. It may be primary or secondary in origin and may differentiate later, earlier, or concomitant to external normal protophloem (Patil *et al.* 2014).

Intraxylary protophloem may initiate from procambium (Scott and Brebner 1889, Baranetzky 1900, Kennedy and Crafts 1931, Zozimo *et al.* 2011), or its derivatives (Esau 1938, Fukuda 1967, Patil and Rajput 2008) on inner margin of procambium. As the secondary growth progress further, secondary intraxylary phloem differentiate from the adjacent parenchyma (Esau 1938, Singh 1943, Patil and Rajput 2008, Patil *et al.* 2009, 2014). Occurrence of intraxylary phloem is characteristic feature of Apocynaceae and is reported in several species of the family but its development in *V. solanacea* is not documented so far. In the present study, *V. solanacea* not only showed both primary and secondary origin of intraxylary phloem but also shows differentiation of secondary xylem in the pith region due to initiation of internal cambium.

*Vallaris solanacea* (Roth) Kuntze (Apocynaceae) is an elegant woody climber cultivated as an ornamental in home gardens for its beautiful and profuse flowering. Anatomical studies on Apocynaceae (Metcalfe and Chalk 1950, Fukuda 1967, Lense *et al.* 2008, 2009) are relatively less as compared to molecular phylogenetic ones (Sennblad and

Bremer 1996, 2002, Potgieter and Albert 2001, Lahaye *et al.* 2005). However, majority of anatomical information comes from the work of Metcalfe and Chalk (1950). Their study merely reported presence of intraxylary phloem while detail studies are lacking on *Vallaris solanacea*. Therefore, present study reports additional features such as occurrence of non-articulated laticifers and production of secondary xylem and phloem by internal cambium which is not documented in the earlier studies. The main objective of the present investigation is to elucidate the structural details of intraxylary phloem, development of internal cambium and differentiation of secondary xylem from internal cambium which is not documented earlier.

## MATERIALS AND METHODS

Samples of the young stems starting from the shoot tip up to maximum thickness (25 mm) available were collected from the *Vallaris solanacea* (Roth) Kuntze, plants growing at the arboretum of the Maharaja Sayajirao University of Baroda, Vadodara (22°19'15.9"N and 73°10' 45.1"E at an altitude of 35.5 m) and in the Botanical Garden of the Government Science College, Gandhi Nagar (23°13′12″ N and 72°40′48″ E). Samples from first visible to 15th internode and thick stems up to 25 mm thickness were collected at various heights from the ground level (i.e. 30 cm, 1m and 2 m) and immediately fixed in FAA (Formalin : Acetic acid: 70% Alcohol, 10:5:85 v/v). After 24 hrs of fixation, they were transferred to 70% alcohol for further processing and storage. Young stem samples were trimmed into 1-2 mm long pieces and dehydrated through tertiary butyl alcohol series and infiltrated in paraffin (Berlyn and Miksche 1976). Transverse and longitudinal sections of thick and woody stems were directly sectioned on Leica (2010R) sliding microtome while serial sections of paraffin embedded blocks were cut with the help of Leica (RM2035) rotary microtome. Sections of 15-20 μm thickness were obtained in transverse, tangential and radial planes and stained with Safranin-Astra blue combination (Srebotnik and Messener 1994). After

dehydration through ethanol-xylene series they were mounted in Dibutyl Phthalate Xylene (DPX).

Length of the sieve tube elements (for both external and intraxylary phloem) was measured directly from the tangential longitudinal sections while length and width of the vessel elements and xylem fibres was measured from macerated material. For maceration of xylem, thin slices of secondary xylem adjacent to the cambium was obtained with razor blade and treated with Jeffrey's fluid (Berlyn and Miksche 1976) at 55-60°C for 36-48 hrs. Macerated material was gently washed with tap water and stained with 1% aqueous solution of Safranin and temporary preparation of slides was used for the measurements. Thirty measurements were taken randomly to obtain mean and standard deviation. Important results were micro-photographed using Leica DM 2000 trinocular research microscope attached with Leica DFC295 fire wire digital camera.

## RESULTS

**Anatomy of young stem and development of intraxylary protophloem :** Young stems were circular in outline and covered with single cell layered thin walled epidermal cells (Plate 1A) that is covered with relatively thick cuticles in relatively thick stems (Plate 1B). Two to three layered oval to polygonal thin walled parenchymatous hypodermis lies below the epidermis. Several cells wide cortex between the hypodermis and pericycle is composed of thin walled, more or less isodiametric, compactly arranged parenchymatous cells (Plate 1B). Inside to the pericycle, procambium initiate in third visible internode (Plate 1C) from the promeristem. At this stage, cluster of two to three external protophloem are also visible. Development of intraxylary protophloem initiate in the fourth visible internode and phloem strands becomes more distinct in fifth internode (Plate 1D). At this stage one or two protoxylem elements (Plate 1D) appeared. In 6th internode well differentiated intraxylary phloem strands are distinct at this stage. Some strands of intraxylary phloem deeply situated in the pith
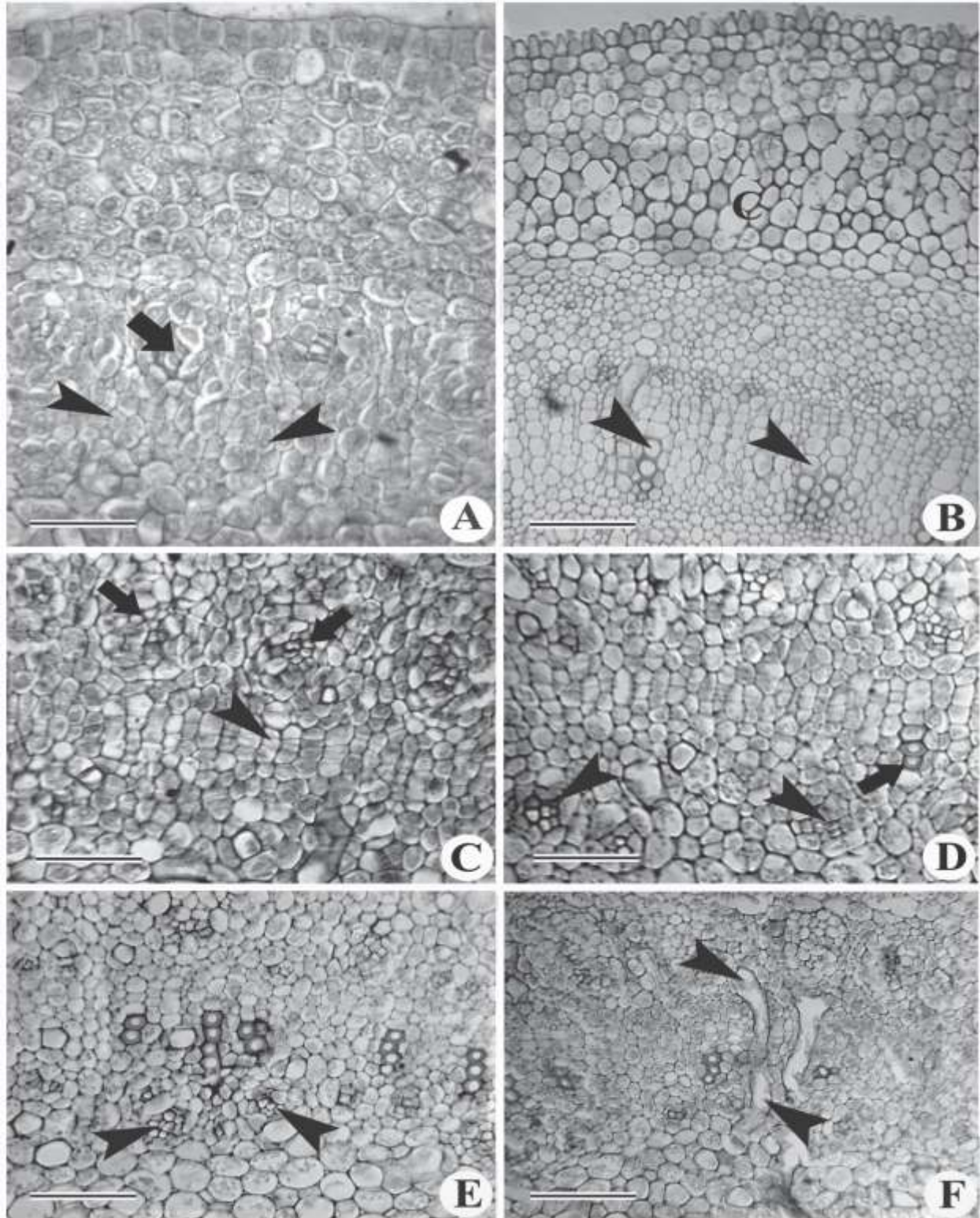
**Plate 1 (A-F) : Transverse view of young stem of *Vallaris solanacea* showing various stages of development. A :** Initiation of procambium (arrowheads) and development of normal external protophloem (arrow) in 3rd internode. Note that initiation of intraxylary phloem is yet to be started. **B :** Structure of young stem showing indistinct pericycle, relatively thick cuticle and structure of cortex. Arrowheads indicate differentiating metaxylem elements. **C :** Initiation of procambium in 3rd internode (arrowhead). Note that differentiation of intraxylary phloem is yet to initiate while arrows showing normal external phloem. **D :** Initiation of intraxylary phloem differentiation in 5th internode (arrowheads). Arrow indicates differentiating protoxylem element. **E :** Well differentiated intraxylary phloem strands in the 6th internode (arrowheads). **F :** Laticifers differentiated in the cortex anatomies with laticifers in pith (arrowhead). **Scale bar :** A, C, D = 75 µm, B-F = 150 µm
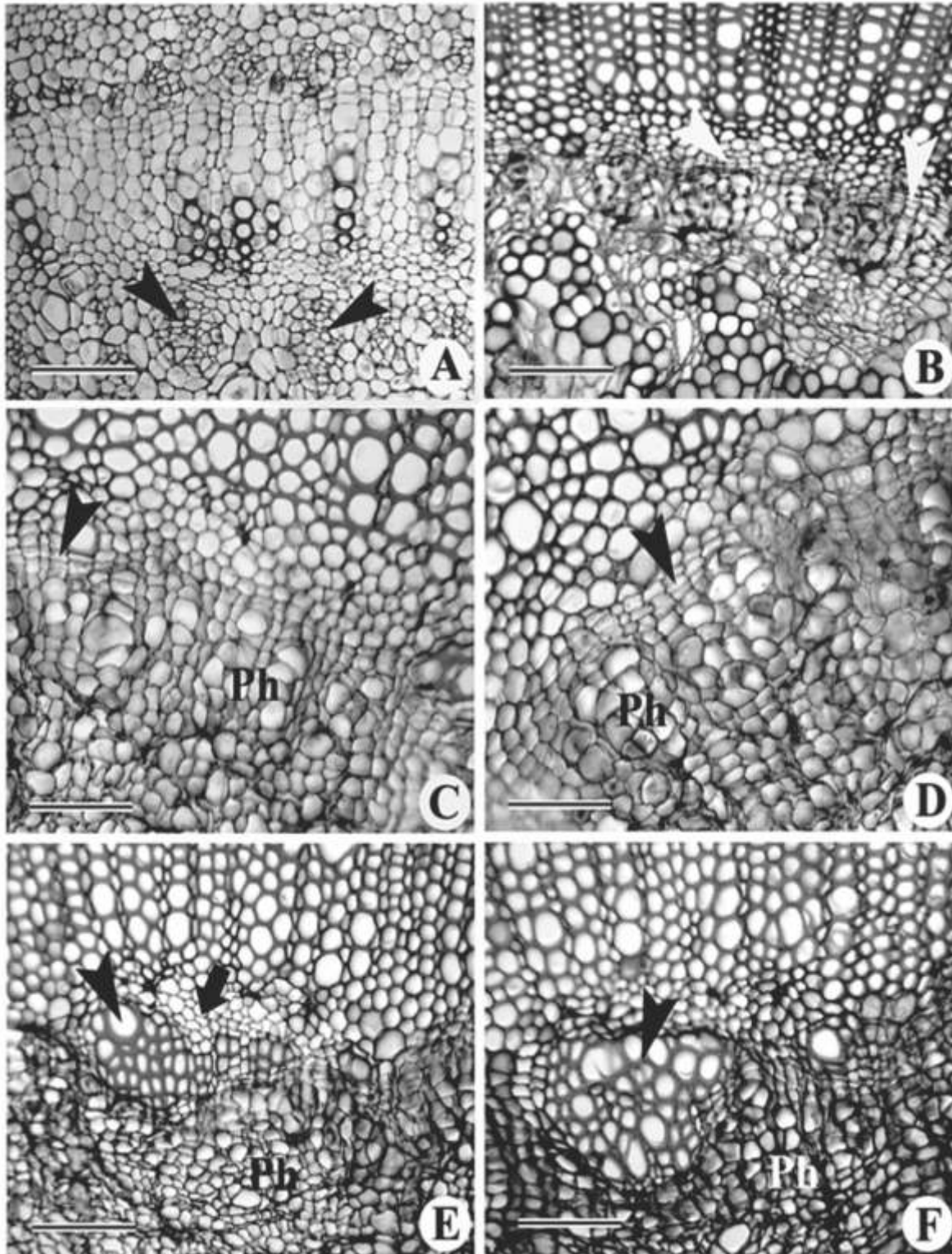
**Plate 2 (A-F ) : Transverse view of stem of *Vallaris solanacea* with different various stages of development of intraxylary phloem and internal cambium development. A :** Well differentiated intraxylary phloem strands (arrowheads) in 9th internode. **B :** Initiation of internal cambium (arrowhead) on the inner margin of protoxylem. **C :** A relatively thick (5-6 mm) stem showing intraxylary phloem (Ph) formed by internal cambium (arrowhead). **D :** Internal cambium (arrowhead) in thick stems. Note the radial arrangement of meristematic cells and differentiation of only phloem elements (Ph) from the cambium. **E :** Bidirectional activity of internal cambium. Note the xylem (arrowhead) and phloem (Ph) from the internal cambium. Arrow indicates pith margin. **F :** Bidirectional activity of internal cambium. Note the xylem (Xy) and phloem (arrowhead) from the internal cambium. **Scale bar :** A, B= 150 µm; C, D = 75 µm; E, F = 125 µm
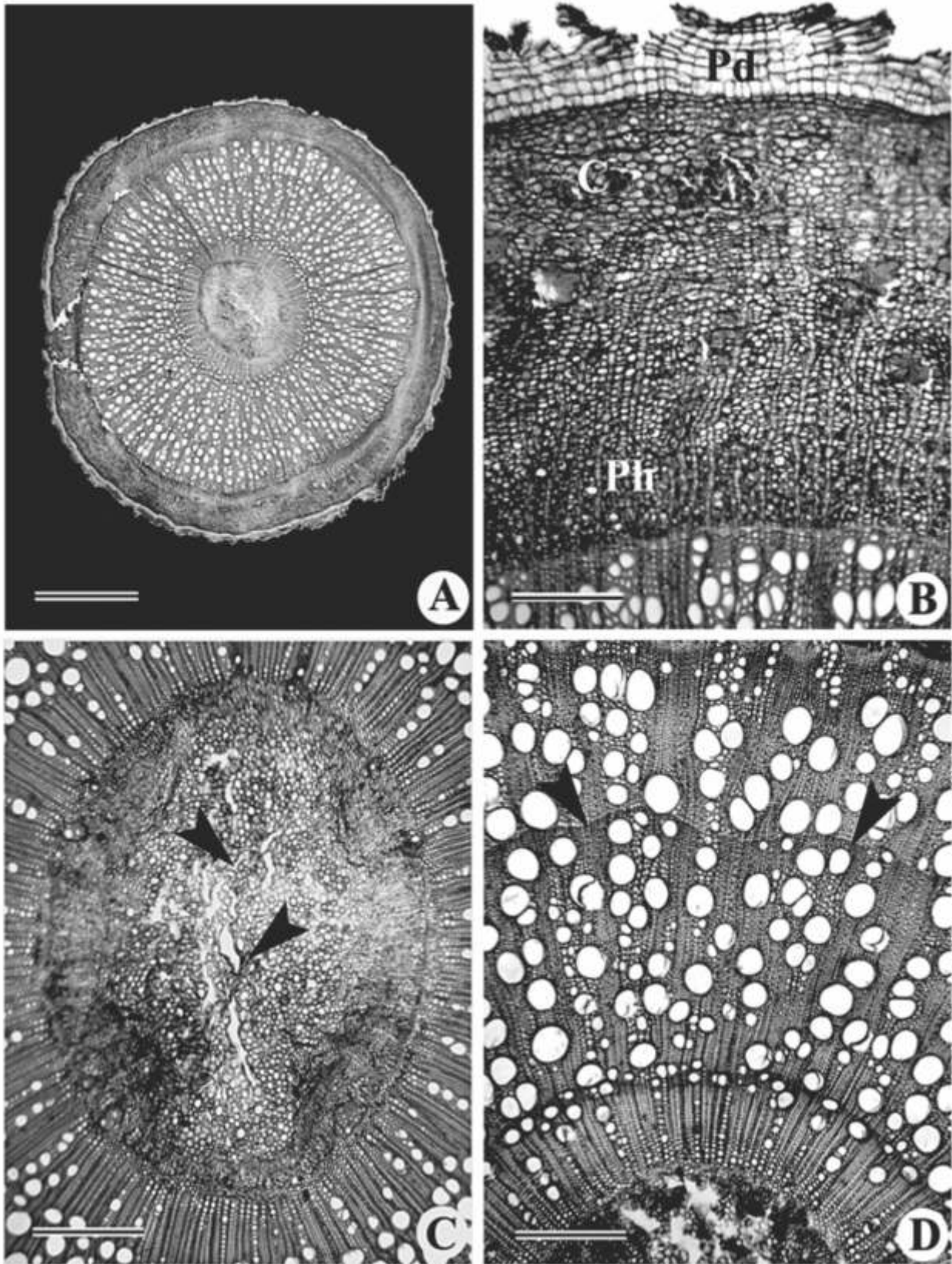
**Plate 3 (A-D) : Transverse view of mature stem of *Vallaris solanacea* A :** Structural outline of 25 mm thick stem. **B :** Mature stem showing periderm (Pd), cortex (C) and secondary phloem (Ph). **C :** Pith portion of mature stem showing obliteration of parenchyma cells (arrowheads). **D :** Structure of secondary xylem. Arrowheads indicate distinct growth rings. **Scale bar :** A = 12 mm; B-D = 250 µm
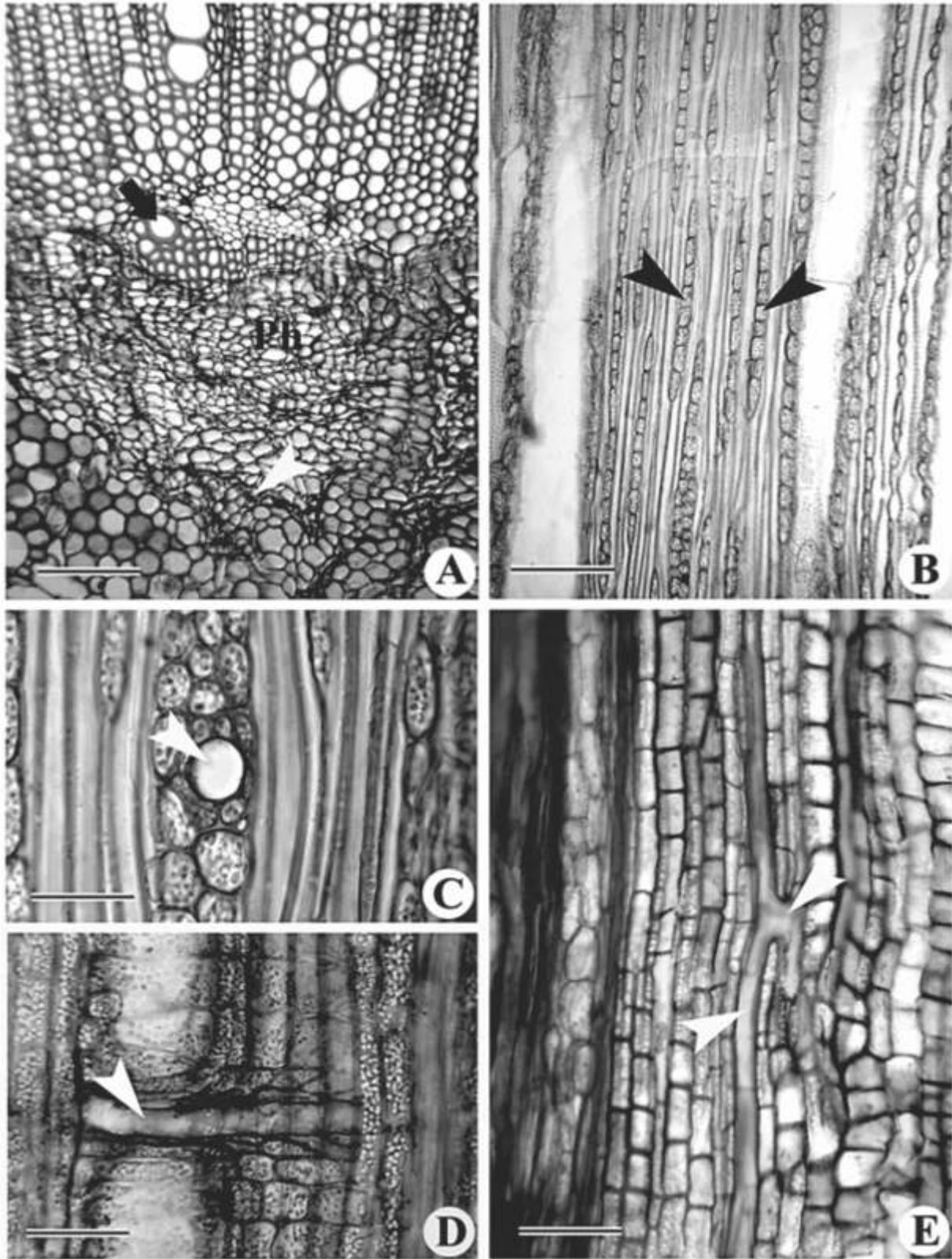
**Plate 4 (A-E) : Transverse (A), tangential (B, C) and radial (D, E) longitudinal view of mature stem of *Vallaris solanacea*. A
:** Part of the mature stem showing earlier formed secondary xylem and pith with secondary phloem (Ph), secondary xylem
(arrow) and obliterated non-conducting phloem (arrowhead). **B :** Composition of secondary xylem. Note the uniseriate rays
(arrowheads). **C :** Xylem ray showing laticifer (arrowhead) in tangential view. **D :** Radially arranged ray showing radially
arranged laticifer (arrowhead). **E :** Portion of the pith showing laticifer (arrowheads). **Scale bar :** A, B = 150 µm, C= 50 µm, D =
75 µm, E = 100 µm

region indicating its probable origin from pith cells because other surrounding cells are morphologically different from the cells that differentiate into intraxylary protophloem (Plate 1E). Several vascular bundles that are composed of one to two protoxylem elements and cluster of three to four protophloem elements on either side of the procambium in fifth internode. Some of the cells in cortex and pith also begin to differentiate into laticifers. As the plant grow further, laticifers differentiated in cortex and pith form anastomosing network (Plate 1F). Intraxylary metaphloem differentiate in ninth internode and the vascular bundles become distinctly visible with enlarging metaxylem elements. Subsequently, small segments of interfascicular cambium initiate between adjacent vascular bundles and complete ring of vascular cambium is established.

**Development of interxylary secondary phloem :** As the growth progress further several strands of intraxylary phloem are distinctly visible in 9th internode (Plate 2A) while additional elements of secondary phloem are added by the adjacent parenchyma cells. Initially, the intraxylary phloem is distributed as distinct pockets but with the increase in age and stem diameter, they form a complete ring on the inner margin on the protoxylem (Plate 2B). When the stems are about 3-5 mm thick, non-conducting interxylary protophloem began to collapse and observed as small patches of crushed cells. As the secondary growth progress further collapse and crushing of secondary interxylary phloem formed in the beginning of secondary growth is also observed. Gradually more and more number of phloem cells is crushed due to addition of new phloem derivatives. At this stage, some of the pith cells also show obliteration and dilation while other cells become thick walled and take safranin indicating deposition of lignin. As the stem increase in thickness, more number of intraxylary phloem elements differentiates into quantifiable amount of phloem in 6-8mm thick stems (Plate 2C).

In 15-18 mm thick stems, small pockets of

thin walled parenchymatous cells sandwiched between protoxylem and intraxylary phloem divide tangentially (Plate 2C, D) to form radially arranged meristematic cells appearing like cambium (referred here as internal cambium). Gradually, internal cambium extends tangentially and forms 3-4 arcs of the internal cambium (Plate 2D) while in some of the samples a complete ring was observed. Initially these segments became functionally unidirectional and form quantifiable amount of intraxylary phloem centripetally (Plate 2C, D). It is composed of sieve tube elements, companion cells, axial and ray parenchyma cells. Sieve tube elements produced by internal cambium are 172-267 (179 + 4.93) µm in length and 15 to 22(17.5 + 0.49) µm in width. Soon after, it starts dividing bidirectionally and began to differentiate secondary xylem centrifugally that appears as small islands of thick walled lignified elements (Plate 2E, F) embedded at the margin of pith. The secondary xylem formed from the internal cambium is mostly composed of vessels and tracheids (Plate 2F) while fibres and parenchyma cells are rare or almost nil. Wide vessels formed in this xylem are 29 to 60µm in diameter.

**Anatomy of mature stem :** Fully grown thick stems are circular in outline (Plate 3A), in which the epidermis is replaced with 4-6 layered cork cells (Plate 3B). Compared to young stems, several cells wide cortex show tangentially flattened parenchyma cells while endodermis and pericycle is indistinct. On the inner side of pericycle lies crushed protophloem and secondary phloem. In earlier formed phloem, sieve elements are completely collapsed and indistinguishable while axial parenchyma cells showed radial and tangential expansion to occupy the empty space formed in response to sieve elements obliteration. This region of secondary phloem also showed randomly distributed isolated or group of sclereids, which are found absent in later formed phloem (Plate 3B). An external secondary phloem is composed of sieve elements, companion cells, axial and ray parenchyma cells. Sieve tube elements are 316-422 (369 + 4.43) µm in length and 17-29

(23.5 + 1.93) μm in width. In pith region most of the cells undergo obliteration (Fug. 3C) while some of the cell undergo lignification and become thick walled.

The secondary xylem was diffuse porous (Plate 3B) with distinct growth rings and was composed of wide and fibriform vessels, tracheids, fibres, axial and ray parenchyma cells (Plate 3D). Axial parenchyma scanty paratracheal and sometime forms incomplete sheath around the vessel. Vessels were solitary, oval to circular in outline and possessed simple perforation plate on transverse end walls and showed dimorphism i.e. distinctly wide and fibriform vessels intermixed with vessels having intermediate tangential diameter (Plate 3D). At this stage stem, show quantifiable development of intraxylary phloem while earlier formed phloem undergo obliteration (Plate 4A). Wider vessels were 297 to 366 (320 + 6.85) μm in length and 89 to 168(126 + 4.35) μm in diameter with alternate bordered pits on lateral walls. Fibriform vessels were exceptionally narrow and appear like imperforated tracheids except presence of sub-terminal simple perforation plate. They were measured about 309 to 388 (348 + 3.63) μm in length and 22 to 37 (28 +0.93) μm in diameter. Rays mostly uniseriate (Plate 4B) while biseriate rays are observed occasionally, they were thin walled and measuring from 431 to 871 (651 + 23.93) μm in height. Length of libriform fibres measured from 633 to 772 (689 + 16.28) μm, non-septate with simple pits arranged slightly oblique to main axis (Plate 4B). As mentioned earlier, radially arranged laticiferes are also observed in the xylem rays (Plate 4C, D).These laticifers are more or less similar to ray cell diameter (Plate 4D) and form anastomosing network. Such laticifers are also observed in the pith region (Plate 4E) which may be branched or unbranched.

## DISCUSSION

Climbers/vines/lianas are group of plants that are incapable to stand erect after certain growth and are dependent on external support (Wyka *et al.* 2013). Lack of self-supporting ability is associated with the allocation of small fraction of biomass to mechanical tissue, while greater amount of biomass is allocated to vertical growth to cope up with the competition for above ground and underground resources. Therefore, their stem diameter is narrow and drastically reduced as compared to self-supporting plants. In doing so they are able to expose their large surface area of the leaves to sunlight (Wyka *et al.* 2013). Their water conducting system is also modified accordingly to supply water to leaf biomass by forming vessels with large lumen diameter. Beside these changes they also show formation of successive cambia, interxylary and intraxylary phloem as an additional path way to conduct the photosynthate.

Being a non-self-supporting species, *Vallaris solanacea* lacks successive cambia and interxylary phloem but development of interxylary phloem is observed from the primary growth of the plants. Intraxylary phloem is the strands of phloem located at the adaxial end of protoxylem of vascular bundles of many dicots. It may be primary in origin i.e. from procambium (Zozomo *et al.* 2011), from procambial derivatives (Patil *et al.* 2009, 2014), from immature pith cells or it may be secondary in origin i.e. by dedifferentiation of mature pith cells (Fukuda 1967; Patil *et al.* 2014). Study of the literature indicates that origin of intraxylary phloem differs from species to species. Intraxylary phloem in *Valleris solanacea* shows its origin in both primary and secondary ways. The intraxylary protophloem develops from procambial derivatives while additional secondary intraxylary phloem develops from the adjacent parenchyma cells located at the periphery of pith. Similar pattern of primary intraxylary protophloem development has been reported by Fukuda (1967), Mikesell and Schroeder (1984), Patil *et al.* (2009, 2014). Available literature also indicates that intraxylary protophloem may occur earlier, after or concomitant to external normal phloem (Baranetzky 1900, Esau 1938, 1969, Singh 1943, Fukuda 1967, Bonnemain 1972, Kuo and Pate 1981, Patil and Rajput 2008, Patil *et al.* 2009, 2014, Zozimo *et al.* 2011). In *V. solanacea* development of normal external

protophloem precedes that of intraxylary phloem. Similar origin has also been recorded in *Pharbitis nil* (Mikesell and Schroeder 1984).

There are few reports in which initiation of cambium between protoxylem and intraxylary phloem is known (Patil *et al.* 2014) which is referred as internal cambium (Patil *et al.* 2009). However, no exclusive documented list of genera is available in which they occur (Carlquist 2013). In the present study thick stem samples develop internal cambium, which is initially unidirectional and form only phloem elements. Later it becomes bidirectional and form secondary xylem abaxially and secondary phloem adaxially. Formation of intraxylary secondary phloem may be alternative pathway to provide conductive safety for photosynthate and protection from external injury like physical and insect herbivory. On the other hand, secondary xylem from internal cambium may be acting as additional path for water supply and mechanical support, since climbing species possess narrow stem diameter to conduct the photosynthate and water to large volume of leaf area. The length and diameter of the sieve tube elements formed by the internal cambium may be relatively more than the external normal phloem elements (Patil *et al.* 2014). In the present study, the length and width of the intraxylary phloem is relatively less than the external phloem. However, studies on dimensional details on the products of internal cambium are less and further studies are needed on more number of species. Development of secondary xylem and phloem from internal cambium may also help to replace the cavity formed in response to collapse of pith cells. Role of intraxylary phloem in long distance translocation of photosynthate has been well established by using carbon isotope (14C) in different species of Solanaceae by Bonnemain (1968, 1972). Further studies are warranted by using recent tools because Turgeon and Oparka (2010) and Zhang *et al.* (2010) demonstrated that beside photosynthate, they also translocate proteins, amino acids, macromolecules and a wide range of unidentified secondary metabolites. Therefore, further studies are warranted to confirm its role by using recent tools and techniques.

A single ring of cambium remains functionally active and forms secondary xylem internally and phloem externally. The secondary xylem is composed of wide and fibriform vessels, tracheids, fibres, axial and ray parenchyma cells. Occurrence of wide vessels and fibriform vessels is a characteristic to climbing habit. Wider vessels are associated with large volume of water in relatively narrow stem diameter while fibriform vessels are resistant to air embolism (Ellmore and Ewers 1985, Carlquist 2001, Rajput *et al.* 2008, 2013). Abundance of thin walled parenchyma either in the form of thin walled patches or as conjunctive tissue in the stems of climbing plants reduces the stem stiffness to provide flexibility against stem torsion (Lowell and Lukansky 1986, 1990, Carlquist and Hanson 1991, Patil *et al.* 2011, Rajput *et al.* 2008, 2013). Beside stem flexibility, parenchyma cells play crucial role in storage of reserved food material and water (Patil *et al.* 2011). As suggested by Carlquist (1985, 2001) and Mooney and Gartner (1991), starch might be used as energy source for growth fluxes and restoration of injured tissues. In contrast, some authors suggest that starch is hydrolysed into sugar that may be carried into the vessels, that increases the osmotic pressure for pulling water into vessels (Araujo and Costa 2006). According to earlier reports (Carlquist 1985, 2001, Mooney and Gartner 1991) this mechanism might be used to recover air embolism in vessels.

Occurrence of growth rings in tropical plants and particularly in climbing species has not been much appreciated due to more or less uniform climatic conditions. As shown in Plate 3D, *V. solanacea* shows distinct growth rings. Seasonal cambial activity and formation of growth rings has been well studied in trees of both tropical and temperate species but similar information on climbing species is lacking. Few efforts have been made on this line by earlier workers and showed presence

of growth rings in perennial climbing species from various families (Baas and Schweingruber 1987, Gasson and Dobbins 1991, Dos Santos 1995, Brandes 2007, Lima *et al.* 2010, Brandis *et al.* 2015). Beside distinct growth rings, occurrence of non-articulated, unbranched laticifersin the xylem rays is unique feature of *V. solanacea.* Presence of such laticifersin the secondary xylem of Euphorbiaceae, Apocynaceae, Asclepiadaceae and Moraceae has been reported by Metcalfe and Chalk (1983) and Evert (2006). According to Rudall (1989), ray initials of the vascular cambium in *Croton conduplecatusare* occasionally converted into laticifer initials which differentiate into non-articulated laticifers in phloem rays. However, in the present study they differentiate in the rays of secondary xylem and may be thin walled and non-lignified. In minority of instances, they may undergo lignification and develop very thick secondary walls (Dressler 1957, Carlquist 1996), as in case of *Euphorbia abdelkuri* (Rudall 1987). Their presence is also reported in different tree species of Apocynaceae (Sidiyas and Baas 1998) but in *V. solanacea* it is reported for the first time. In the present study also, laticifers in the xylem rays adjacent to the cambium are thin walled but in earlier formed secondary xylem they were thick walled and become lignified.

In conclusion, secondary growth in the stems of *Vallaris solanacea* is achieved by single ring of vascular cambium. Occurrence of distinct growth rings in the secondary xylem indicates marked seasonal activity of cambium. Intraxylary protophloem initiates in fourth internode from the procambial derivatives while intraxylary secondary phloem develops from the adjacent parenchyma and subsequently by the activity of internal cambium. The internal cambium which was functionally unidirectional soon becomes bidirectional and produce xylem centrifugally and phloem centripetally. Formation of secondary xylem and phloem from the internal cambium may serve as additional and safer alternate pathway for the conduction of water and photosynthate respectively.

## REFERENCES

Araujo GUC and Costa CG 2006 Cambial variant in the stem of *Serjania corrugata* (Sapindaceae). *IAWA Journal* **27(3)** 269–280.

Baas P and Schweingruber FH 1987 Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. *IAWA Journal* **10(2)** 161–169.

Baranetzky J 1900 Recherchessur la faisceauxbicollatéraux. *Annales des Sciences Naturelles Botanique* **12** 262–332.

Berlyn GP and Miksche JP 1976 *Botanical microtechnique and cytochemistry.* The Iowa State University Press, Ames, Iowa.

Bonnemain JL 1968 Transport du 14C assimilé chez les Solanacées. *Revue Générale de Botanique* **75** 579–610.

Bonnemain JL 1972 Transport chez la plantesupérieure, *PhysiologieVegetale* **10** 697–729.

Brandes AFN 2007 *Anatomia do lenho e dendrocronologia de lianas da famı́lia Leguminosaeocorrentesna* Mata Atlantica. Dissertation, Instituto de Pesquisas Jardim Botaˆnico do Rio de Janeiro, Brazil.

Brandis AFN, Lisi CS, da Silva LDSAB, Rajput KS, Barros CF 2015 Seasonal cambial activity and wood formation in trees and lianas of Leguminosae growing in the Atlantic Forest : a comparative study. *Botany* **93** 211–220.

Carlquist S 1985 Observations on functional wood histology of vines and lianas : vessel dimorphism, tracheids, vasicentric tracheids, narrow vessels, and parenchyma. *Aliso* **11** 139-157.

Carlquist S 1996 Wood, bark and stem anatomy of New World species of *Gnetum. Botanical Journal of the Linnaean Society*, **120** 1–19.

Carlquist S 2001 *Comparative wood anatomy*

*: systematic, ecological and evolutionary aspects of dicotyledon wood.* (2ⁿᵈEd) Springer-Verlag, Berlin.

Carlquist S 2013 Interxylary phloem: Diversity and functions. *Brittonia* **65(4)** 477-495.

Carlquist S and Hanson MA 1991Wood and stem anatomy of Convolvulaceae : A survey. *Aliso* **13** 51–94.

Dos Santos G 1995 *Wood anatomy, chloroplast DNA and flavonoids of the tribe Bignonieae (Bignoniaceae).* Ph D thesis, University of Reading, Reading, UK.

Dressler R 1957 The genus *Pedilanthus* (Euphorbiaceae). *Contributions from the Gray Herbarium of Harvard University* **182** 1–188.

Ellmore GS and Ewers FW 1985 Hydraulic conductivity in trunk xylem of elm, *Ulmus americana. IAWA Journal* **6** 303–307.

Esau K1938 Ontogeny and structure of the phloem of tobacco. *Hilgardia* **11** 343–424.

Esau K 1969 The phloem. In : *Encyclopedia of plant anatomy,* vol. 2. Gebr. Borntraeger, Berlin.

Evert RF 2006 *Esau's plant anatomy. Meristems, cells and tissues of plant body : their structure, function and development* (3ʳᵈedn). John Wiley and Son Inc. Holbrooke, New Jersey.

Fukuda Y 1967 Anatomical study of the internal phloem in the stems of dicotyledons with special reference to its histogenesis. *Journal of Faculty of Science, University of Tokyo* III **9** 313–375.

Gasson P and Dobbins DR 1991 Wood anatomy of the Bignoniaceae, with a comparison of trees and lianas. *IAWA Journal* **12(4)** 389–417.

Hartig T 1854 Über die Querscheide wändezwischen den einzelnen Gliedern der Siebröhrenin *Cucurbita pepo. Bot Zeitung* **12** 52–54.

Hanstein J 1864 *Fie Milchsaftgefäße und die verwandten Organe der Rinde*, Berlin.

Kennedy PB and Crafts AS 1931 The anatomy of *Convolvulus arvensis*, wild morning glory orfield bind weed. *Hilgardia* **5** 591–622.

Kuo J and Pate JS 1981 Intraxylary (medullary) phloem in Macrotyloma uniflorum Lam. (Fabaceae). *Annals of Botany* **48** 403–406.

Lahaye R, Civeyrel L, Speck T and Rowe NP 2005 Evolution of shrub-like growth forms in the lianoid subfamily Secamonoideae (Apocynaceae *s.l.*) of Madagascar: phylogeny, biomechanics, and development. *American Journal of Botany* **92(8)** 1381–1396.

Lens F, Endress ME, Baas P, Jansen S and Smets E 2008 Wood anatomy of Rauvolfioideae (Apocynaceae) : a search for meaningful non-DNA characters at the tribal level. *American Journal of Botany* **95(10)** 1199–1215.

Lens F, Endress ME, Baas P, Jansen S and Smets E 2009 Vessel grouping patterns in subfamilies Apocynoideae and Periplocoideae confirm phylogenetic value of wood structure within Apocynaceae. *American Journal of Botany* **96(12)** 2168–2183.

Lima AC, Pace MR and Angyalossy V 2010 Seasonality and growth rings in lianas of Bignoniaceae. *Trees* **24(6)** 1045-1060.

Lowell C and Lucansky TW 1986 Vegetative anatomy and morphology of *Ipomoea hederifolia* (Convolvulaceae). *Bulletin of the Torrey Botanical Club* **113** 382–397.

Lowell C and Lucansky TW 1990 Vegetative anatomy and morphology of *Ipomoea quamoclit* (Convolvulaceae). *Bulletin of the Torrey Botanical Club* **117** 232–246.

MetcalfeCR and Chalk L 1950 *Anatomy of dicotyledons*. Clarendon Press, Oxford.

Metcalfe CR, Chalk L1983 *Anatomy of dicotyledons II*. Clarendon Press, Oxford.

Mikesell J and Schroeder AC 1984 Internal phloem development in *Pharbitis nil* (L.) Choisy (Convolvulaceae). *Botanical Gazette*, **145** 196–203.

Mooney HA and Gartner BL 1991 Reserve economics of vines. In: PutzFE, MooneyHA

(eds) *The biology of vines*. Cambridge University Press, Cambridge.

Patil VS and Rajput KS 2008 Structure and development of inter- and intraxylary phloem in *Leptadenia reticulata* (Retz.) W. & A. (Asclepiadaceae). *Polish Botanical Journal* **53** 5-13.

Patil VS, Rao KS and Rajput KS 2009 Development of intraxylary phloem and internal cambiumin *Ipomoea hederifolia* (Convolvulaceae). *Journal of the Torrey Botanical Society* **136** 423–432.

PatilVS, Marcati CR and Rajput KS 2011 Development of intra- and interxylary secondary phloem in *Cocciniaindica* (Cucurbitaceae). *IAWA Journal* **32(4)** 475–491.

Patil VS, Koyani RD, Sanghvi GV and Rajput KS 2014 Structure and development of internal phloem in *Solanum pseudocapsicum* (Solanaceae). *IAWA Journal* **35(1)** 1-11.

Potgieter K and Albert VA2001 Phylogenetic relationships within Apocynaceaes. l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. *Annals of the Missouri Botanical Garden* **88** 523–549.

Rajput KS, Raole VM and Gandhi D 2008 Radial secondary growth and formation of successive cambia and their products in *Ipomoea hederifolia* L. (Convolvulaceae). *Botanical Journal of the Linnaean Society* **158** 30–40.

Rajput KS, Patil VS and Rao KS 2013 Wood anatomy and development of interxylary phloem in *Ipomoea hederifolia* Linn., (Convolvulaceae). *Journal of Plant Growth Regulation* **32** 654-662.

Rudall PJ 1987 Laticifers in Euphorbiaceae-A conspectus. *Botanical Journal of the Linnaean Society* **94** 143–163.

Rudall P 1989 Laticifers in vascular cambium and wood of *Croton* spp. (Euphorbiaceae). *IAWA Journal* **10** 379–383.

Scott DH and Brebner G 1889 On the anatomy and histology of *Strychnos*. *Annals of Botany* **3** 275–304.

Sennblad B and Bremer B 1996 The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbcL*data. *Plant Systematics and Evolution* **202** 153–175.

Sennblad B and Bremer B 2002 Classification of Apocynacea*s. l.* according to a new approach combining Linnaean and phylogenetic taxonomy. *Systematic Biology* **51** 389 – 409.

Singh B 1943 Origin and distribution of inter- and intraxylary phloem in *Leptadenia*. *Proceedings of Indian Academy of Sciences B* **18** 14–19.

Sidiyas A and Baas P 1998 Ecological and systematic wood anatomy of *Alstonia* (Apocynaceae). *IAWA Journal* **19(2)** 207–229.

Solereder H 1908 *Systematic anatomy of the dicotyledons*. Vol. 2. Clarendon Press, Oxford, UK.

Srebotnik E and Messener K 1994 A simple method that uses differential staining and light microscopy to assess the selectivity of wood delignification by white rot fungi. *Applied and Environmental Microbiology* **60** 1383-1386.

Turgeon R and Oparka K 2010 The secret phloem of pumpkins. *Proceedings of National Academy of Sciences (USA)* **107** 13201–13202.

Vesque J 1875 Mémoiresurl' anatomie comparée de l'écorce. *Annales des Sciences Naturelles Botanique* **2** 82–198.

Wyka TP, Oleksyn J, KarolewskiP and SchnitzerSA 2013 Phenotypic correlates of the lianescent growth form : a review. *Annals of Botany* **112** 1667–1681.

Zhang B, Tolstikov V, Turnbull C, Hicks LM and Fiehn O 2010 Divergent metabolome and proteome suggest functional independence of dual phloem transport systems in cucurbits. *Proceedings of National Academy of Sciences (USA)* **107** 13532–13537.

Zozimo E, Tamaio N and Vieira RC 2011 Development of intraxylary phloem in the stem of *Combretum rotundifolium* (Combretaceae). *IAWA Journal* **32** 14–24.