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 centrifugally due to 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 multiserial 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 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 (Hansein 1864, Scott and Brebner 1889, Solereder 1908, Esau 1938, 1969, Mikescell 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 (Metcalf 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 the 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 (Metcalf and Chalk 1950, Fukuda 1967, Lense et al. 2008, 2009) are relatively less as compared to molecular phylogenetic ones (Semblad 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, 1 m 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 Mikesche 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 Mikesche 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 protoxylem: 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 protoxylem are also visible. Development of intraxylary protoxylem 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
Stem Anatomy and Development of Intraxylary Phloem in *Vallaris solanacea* (Roth) Kuntze (Apocynaceae)

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 a 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 grows 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 progresses 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 progresses 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
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 Vallaris 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
Stem Anatomy and Development of Intraxylary Phloem in *Vallaris solanacea* (Roth) Kuntze (Apocynaceae)

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 photosynthesize 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 photosynthesize 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 fibriiform vessels, tracheids, fibres, axial and ray parenchyma cells. Occurrence of wide vessels and fibriiform vessels is a characteristic to climbing habit. Wider vessels are associated with large volume of water in relatively narrow stem diameter while fibriiform 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 laticifers in the xylem rays is unique feature of V. solanacea. Presence of such laticifers in 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 conduplicateus are 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.

The authors are thankful to Science and Engineering Research Board (SERB), Government of India for financial support.

REFERENCES


Carlquist S 2001 *Comparative wood anatomy*
Stem Anatomy and Development of Intraxylary Phloem in *Vallaris solanacea* (Roth) Kuntze (Apocynaceae)

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


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


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 9 315–375.


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


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


