CHAPTER – VI

Lianas are one of the fascinating groups of plants that intelligently evolved and adapted to the climbing habit. They are dependent on an object or host to climb on the external support to get mechanical assistance for vertical development towards the light since they are non-self-supporting members of unrelated lineages (Putz & Mooney, 1991; Cabanillas & Hurrell, 2012; Sperotto *et al.,* 2020; Souza-Baena *et al.,* 2021; Leme *et al.,* 2021). Lianas (woody climbers) are perennial, woody non-self-supporting members that are dependent on other plants for their support and climbing (Putz, 1984; Putz & Holbrook, 1991; Liang *et al.*, 2010). They increase their stem thickness by various means by developing the cambial variant (Carlquist 1988, 2001) like regular vascular cambium, by developing various growth trajectories like formation of successive cambia phloem wedges, inter-and intraxylary phloem, compound or polystelic condition or fissured stems (Metcalfe & Chalk 1950, 1983; Obaton, 1960; Putz *et al.*, 1991; Isnard *et al.*, 2009; Angyalossy *et al.,* 2015; Schnitzer *et al.,* 2015). Most of the non-self-supporting plants have slender and narrow stems as compared to the given leaf area they possess (Field *et al.*, 2012) but occasionally they may develop various stem conformations.

Throughout the process of evolution from self-supporting habit towards non-self-supporting growth forms, plants have not only transformed their internal structure of conducting elements but also revised the external morphology that helps them to climb or entwine around the supporting object or host (Rowe *et al.*, 2004; Isnard & Silk, 2009). Darwin (1865) for the first time categorized climbing plants into five categories based on their mode of climbing mechanism and modification of aerial organs which work as the specialized structures playing a strategic role in their climbing behaviour as twiner (e.g., *Ipomoea*, *Merremia*, *Convolvulus*, *Cocculus hirsutus*); Leaf climbers (*Clematis gauriana*, *Gloriosa superba*, *Smilax zeylanica*) Tendril climbers (*Passiflora*, *Cucurbits*, *Serjania*); Root climbers (*Vitis*, *Epipremnum aureum* [money plant], *Ficus pumila*); Hook climbers (*Artabotrys odoratism*, *Ancistrocladus benomensis*, *Calamus*) (*cf*. Isnard & Silk, 2009).

The climbing habit evolved by achieving stem flexibility and several structural modifications in the mechanical tissue (Rowe & Speck, 1996, 1998, 2005; Rowe *et al.,* 2004; Angyalossy *et al.,* 2015; Lekhak *et al.,* 2018). Stem flexibility is accomplished by changing growth patterns and comparatively profusion of parenchyma cells and reduction in the mechanical tissue, wide rays, formation of parenchyma wedges, phloem wedges and by developing various kinds of cambial variants (Obaton, 1969; Carlquist, 1988, 2001; Pace *et al.*, 2009, 2018; Gerolamo *et al.*, 2020). Among these alterations, modification in the secondary growth is one of the ways to advance hydraulic competence and increase stem flexibility (Carlquist, 1988; Ewers & Fisher, 1991; Rowe *et al.*, 2004; Isnard & Silk, 2009; Schnitzer & Bongers, 2011; Rajput *et al.*, 2012, 2013; Lekhak *et al.,* 2018) for climbing or twining on the host that protects the conducting tissues from internal injury during stem torsion (Carlquist, 1988). The narrow stem diameter and altered mechanical tissue of the stems in climbing plants act like a cable and help them to protect the vessel from cavitation while an abundance of axial parenchyma helps them during external and internal injuries (Carlquist, 2001; Rowe *et al.*, 2004; Rowe & Speck, 2005; Isnard & Silk, 2009; Schnitzer & Bongers, 2011; Rajput *et al.,* 2012b, 2013; Lekhak *et al.,* 2018). Alterations in the pattern of secondary thickening in lianas may vary from species to species or may be unique and characteristic to certain tribes and families or may be shared by allied genera (Rajput *et al.*, 2021). Alterations in the structure and composition of the stems of non-self-supporting plants are usually known to occur only after the initiation of the climbing habit (Caballe, 1998; Carlquist, 2001; Rowe *et al.*, 2004; Rowe & Speck, 2005; Isnard & Silk, 2009; Rajput *et al.*, 2012b, 2013; Angyalossy *et al.*, 2015; Lekhak *et al.*, 2018; Chery *et al.*, 2020; Tamaio *et al.*, 2021). During the early stages of secondary growth (i.e., before the development climbing habit), young plants or their branches show typical secondary growth which is characteristic of self-supporting plants (Rowe & Speck, 1996, 2005). Most of the non-self-supporting members show the development of various types of cambial variants that are hypothesized to result from the climbing habit (Carlquist, 1988, 2001; Isnard & Silk, 2009; Angyalossy *et al.*, 2015; Lekhak *et al.*, 2018; Rajput *et al.*, 2021), including the formation of successive cambia. All these cambial variants have been categorized variously by earlier researchers (Schenck, 1893; De Bary, 1984; Pfeiffer, 1926; Metcalfe & Chalk, 1950, 1983; Boureau, 1957; Obaton, 1960; Philipson & Ward, 1965; Carlquist, 1988, 2001). These classifications include extensive monographs (Pfeiffer, 1926), diagrammatic illustrations (Chalk & Chattaway, 1937) or cellular details and outline diagrams of the monograph (Obaton, 1960). Anatomically, these classifications also include, bicollateral vascular bundles, medullary bundles, cortical bundles, intraxylary (internal) phloem, interxylary (included) phloem, concentric rings of xylem alternating with phloem, stem become elliptical, flattened, lobbed, fissured xylem or furrowed in cross-section, stems lobed. Among them, the family Sapindaceae is of special interest and treated separately due to a unique type of cambial variant (referred to as compound xylem i.e., polystelic xylem). With this brief background, the present study attempts to understand the evolution of structural alterations in the growth patterns and structure of the secondary xylem by studying their developmental stages in selected tropical lianas.

Stem samples of various thicknesses (shoot tip to maximum possible thickness available) were collected from the five individuals each of the species growing naturally or under-cultivation. Stem samples of a total of 50 different species belonging to 10 angiosperm families and 2 Gymnosperm families were collected from various bio-geographical locations throughout the country (including some samples from Durban, South Africa and Osa Peninsula, Costa Rica). To study various developmental stages; stems with different diameters starting from shoot tip to mature stems were harvested 10 cm above the ground level and subsequently, samples were collected from every 50 cm interval towards up to the apex. Stem pieces measuring 40-60 mm in length were cut and fixed immediately in FAA after the collection (Berlyn & Miksche, 1976). After 48-72 hours of fixation, samples were transferred to 70 % ethanol for further processing and storage.

For the macro-morphological study, thick stem samples of suitable sizes were trimmed using a sharp blade and allowed to dry for a few minutes and photographed with Canon (DSLR – EOS 1500D) or by using the stereo zoom microscope (Leica S6D & Leica 205C fitted with Leica IC90E camera) and sometimes using a mobile camera (Nokia 7.2). For histological preparations, young and soft stems were processed through a Tertiary Butyl Alcohol (TBA) series, embedded in paraffin wax after infiltration (Berlyn & Miksche, 1976) and sectioned using a Leica RM 2035 rotary microtome. Thick stem samples were sectioned directly on Leica SM 2010R sliding microtome. Transverse, radial and tangential longitudinal sections of 15-20 µm thickness obtained from both, soft and hard samples were dehydrated through an ethanol-xylene series and mounted in DPX after staining with the Safranin-Astra blue combination (Srebotnik & Messner, 1994). Microphotographs of important results were taken using a Leica DME 2000 trinocular photomicroscope with Leica DFC 295 firewire digital camera.

Dimensional details of the secondary xylem derivatives were studied by maceration of the outermost secondary xylem adjacent to the vascular cambium. Small portions of the secondary xylem were sliced into small pieces with a razor blade and treated with Jeffrey's fluid (Johansen, 1940). These xylem slices were incubated at 55-60 ^{o}C for 24-36 hours washed thoroughly with distilled water and stained with 0.5% aqueous safranin. Lengths of the vessel elements and fibres were measured from the macerated material while dimensional details of sieve tube elements, ray height and width (of both xylem and phloem) were measured from the tangential longitudinal sections while vessel diameter (tangential) was measured from the transverse sections as recommended by IAWA committee (1989). Thirty measurements for each cell type were taken randomly to get the mean and standard deviation. Mean and SD was calculated using Microsoft Excel.

To achieve the proposed objectives, fifty two species (including gymnosperm like *Ephedra foliata*, *E. karumanchiana* and *Gnetum ula*) from different families that are dominated with climbing habit were selected to study the alteration in the stem structure in response to climbing habit (Table 1). Histological analysis of all these samples revealed that they were characterized by the presence of one or other type of variant secondary growth. However, some of the members like *Ampelocissus latifolia*, *Cayratia auriculata*, *Cissus quadrangularis*, *C. repanda*, C. *rotundifolia*, *Dalbergia volubilis*, *Cissampelos pareira*, *Coscinium fenestratum*, *Stephania japonica*, *Tinospora cordifolia, T. sinensis, Vitis vinifera* and from gymnosperms *Ephedra foliata* and *E. karumanchiana* showed a regular way of secondary growth with a single ring of the vascular cambium like other eudicots or they may specific one or presence of multiple cambial variants. All these variant secondary growths are categorised into different types such as:

i) *Xylem structure from the transition from self-supporting to climbing habit*: Members like *Arrabidaea candicans* (Bignoniaceae), *Beaumontia jerdoniana*, *Dicranostyles ampla*, *Entada gigas, Entada rheedii, Maripa nicaraguensis*, *Serjania mexicana*, *Strychnos bredemeyeri,* S. *andamanensis* and *Valleris solanacea* showed typical secondary xylem that is characteristic to self-supporting habit before the initiation of climbing habit. After the initiation of climbing, structure of the secondary xylem was altered abruptly and showed typical xylem that is characteristic to the lianescent habit. In contrast, remaining species showed no such distinction in the structure of the secondary as shown by above mentioned species.

ii) *Formation of the secondary xylem in plates*: In the members of Menispermaceae like *Anamirta cocculus, Cissampelos pareira, Cocculus hirsutus, C. pendulus, Coscinium fenestratum, Diploclausia glauscence, Pachygon ovata, Stephania japonica, Tinosporia cordifolia, T. sinensis*, and *Tiliacora racemosa* (including trees like *Cocculus laurifolius*), *Cissus quadrangularis, C. repanda, Vitis vinifera* of Vitaceae and gymnosperm like *Ephedra foliata* and *E. karumanchiana* showed the formation of xylem in radial plates. Formation of such a xylem resulted in response to relatively wide rays and narrow sector of the fascicular sector of the cambium that produces axial elements. Development of xylem in plates was observed in species with regular secondary growth and species with successive cambia.

iii) *The abundance of parenchyma* (*both axial and ray*): The secondary xylem of the plants of the family Fabaceae (*viz. Canavalia gladiata, C. virosa, Entada rheedii, Phaseolus lunatus, Pueraria tuberosa*) and Vitaceae (*viz. Ampelocissus latifolia, Cayratia auriculata, Cayratia trifolia, Cissus quadrangularis, Cissus repanda* and *Tetrastigma bracteolatum*) showed abundance of unlignified axial parenchyma while libriform fibres were restricted around the vessels or distributed as small islands within the parenchymatous background.

iii) *Formation of successive cambia*: Present study revealed the development of successive cambia from various cell types like pericycle parenchyma, cortical parenchyma cells, from the dilating phloem parenchyma (both ray and axial) or both ray and axial parenchyma cells of the xylem. Based on their origin, formation of successive cambia may be categorised as:

a). *Initiation of successive cambia from pericyclic parenchyma or derivatives*: In species like *Antigonon leptopus, Cyratia trifoliata*, *Dicranostyles ampla*, *Maripa nicaraguensis, Moutabea gentry, Tetrastigma bracteolatum* and *Turbina corymbosa* the increase in stem thickness was achieved by forming successive rings of cambia. These cambia were initiated from the pericyclic parenchyma cells. After a short period of secondary growth, parenchymatous cells (likely pericycle) located outside the protophloem acquired meristematic activity and gave rise to the first ring of successive cambium.

b). *Dilatation phloem ray parenchyma and initiation of neo-formed vascular cylinders*: In species like *Hewittia malabarica*, *Phaseolus lunatus* and *Turbina corymbosa* the development of neo-formed vascular cylinders was observed from such a dilatation meristem. Most species in the family Convolvulaceae are characterized by the presence of successive cambia from the pericycle parenchyma while some members complete their lifecycles with a single vascular cambium. Unlike other members of the Convolvulaceae, the additional cambia in *Hewittia malabarica* and *Turbina corymbosa* do not connect to form a complete ring of the successive cambium rather it form individual vascular cylinders.

c) *Initiation of successive cambia from cortical parenchyma*: Among the species investigated, members of Menispermaceae (like *Anamirta cocculus*, *Cocculus hirsutus*, *C. laurifolius*, *C. pendulus*, *Cyclea peltata*, *Diploclisia glaucescens,* *Pachygone ovata* and *Tiliacora racemosa*) and *Rhynchosia pyramidalys* (Fabaceae), the development of successive cambia occurs from the cortical parenchyma cells.

d) *Development of functionally inverse cambia*: Development of inverse cambia is a rare feature and is reported in a few members. In the present study formation of such functionally inverse cambia is reported herewith in *Argyreia elliptica*, *Canavalia gladiata*, *C. virosa*, *Entada rheedii*, *Dicranostylis ampla*, *Phaseolus lunatus*, *Turbina corymbosa* and *Hewittia malabarica*. During the initiation of successive cambia, the parenchyma cells formed before the cambium initiation, cells located on the inner margin of the meristematic zone differentiated into conjunctive tissue, which served as a source for the initiation of inverse cambia in future, whereas cells on its outer margin become the source for the further successive cambia.

e) *The proliferation of xylem rays and formation of ray cambium*: In *Canavalia gladiate*, *C. virosa*, *Hewittia malabarica*, *Phaseolus lunatus*, *Pueraria tuberosa* and *Turbina corymbosa* the marginal ray cells of the multiseriate rays acquired meristematic activity and initially formed only phloem elements. However, as time passed it also showed differentiation of the secondary xylem. Initiation of the ray cambium and differentiation of the xylem and phloem from the ray cambium coincide with the reproductive phase of samples collected in the present study.

iv) *Development of interxylary phloem*: Formation of strands of sieve tubes and associated parenchyma cells embedded within the secondary xylem of the stems and roots was observed in *Canavalia virosa*, *C. gladiata*, *Entada rheedii*, *Hewittia malabarica*, *Leptadenia reticulata*, *L. pyrotechnica*, *Phaseolus lunatus*, *Pueraria tuberosa*, *Strychnos bredemeyeri*, *S. andamanesis* and *Turbina corymbosa*.

v) *Development of intraxylary phloem and intraxylary phloem cambium*: Present study elucidates the development of such phloem in *Beaumontia jerdomiana*, *Campsis radicans*, *Dicranostyles ampla*, *Hewittia malabarica*, *Jacquemontia paniculata*, *Leptadenia reticulata*, *L. pyrotechnica*, *Maripa nicaraguensis*, *Strychnos* *bredemeyeri, Turbina corymbosa, Vallaris solanacea*, and *Wattakaka volubilis*. In all the investigated species formation of intraxylary phloem was observed from the beginning i.e., from the initiation of primary growth except in *Campsis radicans*. Development of such a phloem in this species was observed only after the initiation of secondary growth.

vi) *Development of phloem wedges* (*furrowed xylem*) *and lobbed stems*: Phloem wedges are the discrete variant portions of the secondary xylem that derive from the small segments of the vascular cambium due to shift from the regular pattern and deposit less xylem and more phloem. Formation of such phloem wedges is one of the important strategies of the climbing plants to enhance the stem flexibility against the stem twisting and swinging due to strong wind currents or movement of arboreal animals.

Some of the species investigated in the present study showed a special case of growth pattern which is not been reported so far from the Indian species. In the present two such taxa, (*Serjania circumvallate* and *S. mexicana* of the family Sapindaceae) collected from the Osa Peninsula of Costa Rica are investigated as a special case of growth pattern for the climbing habit. *S. circumvallata*) showed the development of polystelic condition, in which 3-5 additional vascular strands initiated external to the phloem of central vascular cylinder giving an appearance like 4-6 vascular cylinders clubbed together in a single stem. . In contrast, later species (*S. mexicana*) showed phloem wedges like the members of the tribe Bignonieae but the only difference was these wedges were uneven in number followed by it also showed the development of neo-formed cylinders external to the phloem wedges.

Conclusion:

Though, cambial variants are most noticeable in lianas and vines; nevertheless, there are other plant species from completely different habit in which cambial variants exists. The existence of a comparatively tough and unbending secondary xylem during self-supporting stage aids the young shoots to stand upright in search of supportive objects. Plant species in which the shoot portion grows upright for certain height and then search for support usually display such secondary xylem and the xylem characteristics to the climbing growth form starts only after the initiation of climbing on the supporting object. Structure of secondary xylem in climbing species differs considerably from the erect species, which enable stem elasticity and may protect the straight growing stems from twisting, bending and internal injury.

The occurrence of various cambial variants like multiple cambia or various anatomical configurations such as discrete xylem or xylem in successive rings, interxylary and intraxylary phloem, non-circular stems, and furrowed or crenate stem conformation attributed to ecological adaptations that might be in response to the environmental conditions. A mixture of lignified and unlignified tissues that might be important in providing the flexibility to climbing stem tissues, which may permits to twist without causing damage to the secondary xylem, may helpful in storage of photosynthate or that might be very much important during restoring from the injury and damaged tissues.

Production of intraxylary phloem provides potency for a safe way as compared to the outer phloem for the conduction of photosynthate. Due to the presence of narrow stem, when the plants reach to flowering and fruiting stage, there is a great demand of photosynthate for the accurate development of seeds and fruits. Therefore, additional photosynthate conducting tissues are required. In such condition, development of internal cambium may takes place to form quantifiable amount of secondary phloem which may fasten the process of photosynthate conduction. It also facilitates the alternate pathway for transportation of photosynthate and any other metabolites. This may ensure the transportation in case of bark damaged during climbing around the host or by insects like stem borers.

Various functions have been ascribed for the presence of interxylary phloem in plants. It may provide alternate way for transport of phloem sap and ensures the protection of phloem from bark eating insects. Available literature indicates that high temperature leads to non-functioning of conducting phloem. Since, the interxylary phloem islands are surrounded by secondary xylem that protects this phloem from high temperature during summer season and keep the phloem functionally active when the temperature is high during summer. For example in case of *Leptadenia pyrotechnica* which is well adapted to xeric condition, shows the presence of Intraxylary and Interxylary phloem.

The parenchyma abundance in xylem, multiple cambial rings i.e. successive cambia and presence of wide rays, phloem wedges, compound xylem and xylem in plates might be helpful in maintaining of hydraulic integrity despite the stem is fractured due to bending or twisting force along a flexible parenchyma matrix and also helps in development of new cambia or parenchyma during wound healing. More amount of parenchyma cells may offers additional location for the storage of starch that can be utilized during a period of reproductive time or during the harsh environmental conditions. It is also expected that these prevents the water conducting tissues during the twisting around host against torsion and damage. Reduced amount of sclerenchymatous fibres gives stem more flexibility instead of stiffness, fibres are presence only around the vessels which is helpful in protection against rupturing due to hydraulic pressure and twisting force. The presence of Gelatinous fibre islands, long and very thin libriform fibres and fibre tracheids in secondary xylem again gives stems more mechanical strength and flexibility. The presence of vessel dimorphism i.e. wide vessels along with narrow vessels may allows the bulk flow of water in narrow stem diameter; it might be also ensures the conduction during the blockage or inactivation or air embolism of vessel element.

From the histological peculiarities and results obtained by the present investigations, comparing with the plant habit and habitats they are adapted, the environmental conditions they are passing through and comparing with available literatures; it can be concluded that the present study is in agreement with the above said hypothesis since several species in the present study showed formation of various types of cambial variants like internal cambium, interxylary and intraxylary phloem, intraxylary xylem, successive cambia, phloem wedge, flattening or lobed stem, parenchyma abundance, wide rays, xylem in plates and compound xylem; which are also reported in our publications and by earlier researchers in various climbing plants and their significance.