CHAPTER - V

The evolution of climbing habit has altered the ecology in terms of resource and energy cycling and reaction to the environment of plant and forest populations (Heijden *et al.*, 2015). It is one of the most significant developments in the diversification of angiosperms was the evolution of structural variety, also known as heteroxylly (Carlquist, 2009; Feild *et al.*, 2012; Isnard & Field, 2015). This variety in structural diversity will be crucial to the vascular tissues hydraulic, mechanical, and storage processes (Carlquist, 2009; Feild *et al.*, 2012). In instance, lianas have investigated heteroxylly using a wide range of cambial functionality. In general, a bifacial vascular cambium produces phloem centrifugally and xylem centripetally which is a regular and usual type of cambial activity (Evert, 2006; Spicer & Groover, 2010). Nevertheless, in many of the other plants mainly lianas cambial activity diverges sharply from a characteristic development to form a broad range of stem developmental and structural alterations. This includes 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 (Schenck, 1892; Obaton, 1957; Carlquist, 1991; Caballé, 1993). This type of cambial variants also referred as “anomalous secondary growth” is reported to occur in climbing plants that represents a unique habit which is distributed in the angiosperm phylogeny (Spicer & Groover, 2010; Angyalossy *et al.*, 2012). Throughout the Mesangiosperm phylogeny, the evolution of cambial variations has repeatedly occurred, and this is strong support for their adaptive relevance (Spicer & Groover, 2010; Isnard & Field, 2015; Angyalossy *et al.*, 2012).

The appearance and physiology of woody climbers and lianas differ from those of more common plants. Lianas exhibit unusual secondary growth as they grow thicker. There are other examples of variant secondary growths that revealed inconsistencies in the design of stem architecture (Eames & McDaniels, 1953; Sajo & Castro, 2006). Haberlandt (1914) referred these variant growth trajectories as adaptive and non-adaptive unusual secondary growths. Although the exact reason for the occurrence of variant secondary growth is yet to be known, it is thought that physiological or environmental stress is a contributing factor (Joshi, 1935; Dobbins & Fisher, l986; Putz & Mooney, 1991). The cambium modifies its activity in certain areas deposit a mixture of lignified and unlignified tissues that provide flexibility to the climbing stems (Rowe *et al.*, 2004; Isnard & Silk, 2009). Formation of such thick walled lignified tissue intermixed with thin walled unlignified tissues permit climbing plants to twist without causing damage to the secondary xylem (Carlquist, 1988). It is also believed that the variant structures plays an important role in storage of photosynthate (Carlquist, 1988), restoring from the injury and damaged tissues (Carlquist, 1985, 2001; Dobbins & Fisher, 1986; Fisher & Ewers, 1991, 1992; Rajput *et al.*, 2008; Patil *et al.*, 2011; Angyalossy *et al.*, 2015) and also attributed many more ecological significance for the occurrence of variant secondary growth (Eames & McDaniels, 1947; Philipson *et al.*, 1971; Metcalfe & Chalk, 1983; Carlquist, 1988, 2001a; Fisher & Ewers, 1989). According to Kemer (1895) stem flattening occurs due to lateral pressure injuries in the conducting tissues, which these plants avoid by developing stems with a ribbon-like structure. According to Obaton (1960), flattened stems are exclusive to plants that are epiphytic lianas. However, it is not applicable to climbing members.

Throughout the course of angiosperm evolution, several plant families independently developed cambial variants, with certain variants being so distinctive and characteristic to each group and these families may be solely recognized by their stem topographies (Caballe, 1993). For example, some members of the family Fabaceae display asymmetrical growth (Schenck, 1983; Fisher & Ewers, 1992), Sapindaceae shows several steles (Schenck, 1983; Carlquist, 1988), Menispermaceae is characterized by the presence of successive cambia, and Bignoniaceae by forming a phloem wedges and furrowed xylem due to (Dobbins, 1971; Schenck, 1983; Carlquist, 1988). Such growth patterns increase the stem flexibility and protect the mechanically vulnerable tissues in large and lengthy vines (Putz & Holbrook, 1991).

The occurrence of cambial variants attributed to ecological adaptations that are driven by environmental conditions (Carlquist, 1988). Though, cambial variants are most noticeable in lianas and vines; nevertheless, there are other plants species from completely different habit in which cambial variants exist. For example, successive cambia and the primary thickening meristem generate the rings of vascular tissue in beet (*Beta vulgaris*, Chenopodiaceae). This method might be an effective way to give the storage structure innervations. On the other hand, other storage organs, like potatoes or sweet potatoes, have vascular strands dispersed throughout them without subsequent cambia (Rapoport & Loomis, 1986; Carlquist, 2007a; Rajput *et al.*, 2008). In contrast, there are certain species that are not climbing but are characterized by the presence of similar type of cambial variant which is characteristic feature of that family. Few of them are *Cocculus laurifolius* (Menispermaceae; Rajput & Gupta, 2015), *Leptadenia pyrotechnica* (Asclepiadaceae; Gondaliya & Rajput, 2017), *Gallesia integrifolia* (Phytolaccaceae, Rajput *et al.*, 2012) and there are several other examples.

Climbing or non-self-supporting plants are characterized by the presence of cambial variants like successive cambia, functionally inverse cambia, inter-and intraxylary phloem, internal/intraxylary phloem cambium, compound/polystelic stems, phloem wedges and furrowed xylem (Eames & McDaniels, 1947; Philipson *et al.*, 1971; Metcalfe & Chalk, 1983; Carlquist, 1985, 1988, 2001; Dobbins & Fisher, 1986; Fisher & Ewers, 1989, 1991, 1992; Rajput *et al.*, 2008, 2013, 2017, 2021; Patil *et al.*, 2011; Angyalossy *et al.*, 2015; Lekhak *et al.*, 2018; Tamaio *et al.*, 2021).As shown in Table 1, several climbing members of various families that are dominated with climbing habit were investigated histologically in the present study. All the members were characterized by one or other type of variant secondary growth (including *Gnetum ula* from the gymnosperms) except *Ampelocissus latifolia*, *Cayratia auriculata*, *Cissus quadrangularis*, *C. repanda*, C. *rotundifolia*, *Dalbergia volubilis*, *Cissampelos pareira*, *Coscinium fenestratum*, *Stephania japonica*, *Tinosporia cordifolia*, *T. sinensis*, *Vitis vinifera* and from gymnosperms *Ephedra foleata* and *E. karumanchiana*. Above mentioned all the species showed regular secondary growth like other eudicots.

During the primary growth most of the eudicots (including species with cambial variants) show regular pattern of growth i.e., procambium initiate as a small sector at several places and its bifacial activity leads to formation of vascular bundles in the form a ring. Subsequently, these vascular bundles interconnect with the help of interfascicular cambium to form a complete ring of vascular cambium (Eames & McDaniels, 1947; Philipson *et al.*, 1971; Fahn 1972; Rudal 2007; Cutler *et al.*, 2007; Beck 2010; Crang *et al.,* 2018). However, in the present study, members of families like Apocynaceae, Convolvulaceae, Cucurbitaceae and Loganiaceae also showed primary variant growth (intraxylary protoxylem) along with the regular secondary growth (Gondaliya & Rajput, 2016, 2017, 2018; Lekhak *et al*., 2018; Moya *et al.*, 2017, 2019; Rajput *et al.,* 2017, 2018, 2021). After the initiation of the secondary growth, the vascular cambium differentiate xylem centripetally and phloem centrifugally. In the initial stage of the secondary growth, structurally the xylem appeared like self-supporting plants but as the secondary growth progressed, in some of them (not all) composition and structure altered suddenly and showed transition from self-supporting to climbing habit.

***Xylem structure from transition from self-supporting to climbing habit*:** The young shoots of some members like *Arrabidaea candicans* (Bignoniaceae), *Beaumontia jerdoniana*, *Dicranostyles ampla*, *Entada rheedii*, *Maripa nicaraguensis*, *Serjania mexicana*, *Strychnos bredemeyeri*, S. *andamanensis* and *Vallaris solanacea* showed typical secondary xylem of self-supporting habit before the initiation of climbing habit. Such xylem was relatively hard and stiff and possessed relatively narrow vessels, higher number of xylem fibres and parenchyma cells were either absent or minimal. A similar feature of the secondary xylem is reported by Rowe & Speck (1996, 2005) and Rajput *et al.,* (2022). The existence of such a comparatively tough and unbending secondary xylem aids the young shoots to stand upright in search of supportive objects. Therefore, referred to as 'searchers' by Rowe & Speck (2005). 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 protect the straight growing stems from twisting, bending and internal injury (Fischer & Ewers, 1989; Rowe & Speck, 1996; Carlquist, 2001; Speck & Bugert, 2011) due to external factors or swinging of the stems (Rajput, 2015). As compared to climbing condition, the secondary xylem of upright growing shoots and young saplings of all the species investigated revealed presence of thick walled fibres with narrow lumen, vessels with relatively less diameter, uniseriate rays and scanty paratracheal parenchyma.

Structure of the secondary xylem after climbing**:** As mentioned above, after the initiation of the climbing habit, considerable alterations were observed in the structure of secondary xylem. Suddenly, deposition of frequency of the vessel and increased vessel diameter was the notable change while number of axial parenchyma increased and changed from scanty paratracheal to other types including formation of patches/islands of unlignified parenchyma distributed randomly. Subsequently, ray width also increased in most of the members while species like *Arrabidaea candicans*, *Beaumontia jerdoniana* and *Vallaris solanacea* retained uni-biseriate rays while multiseriate rays developed rarely. In contrast, those species that do not show such transition from like above said species showed production of wide vessels with higher frequency, multiseriate rays, abundance of axial parenchyma and relatively less xylem fibres from the beginning of secondary growth. The presence of wider vessels and multiseriate rays compared to self-supporting phase and abundance of axial parenchyma is characteristic to climbing habit (Ellmore & Ewers, 1985; Fisher & Ewers, 1989; Ewers *et al.,* 1991; Rowe & Speck, 1996; Carlquist, 2001; Rajput *et al.,* 2013; Angyalossy *et al.,* 2015; Onyenedum & Pace 2021). Ewers *et al.,* (2015) correlated, increase in vessel diameter and their frequency with the leaf surface area and the amount of water required for transpiration while decreased mechanical tissue helps in increasing the stem flexibility by reducing the stem stiffness. All these features provide stem flexibility for twisting around the host without any disadvantageous effect on conductivity of the secondary xylem (Rowe & Speck, 2005; Angyalossy *et al.,* 2011, 2015). According to Onyenedum and Pace (2021, pp 2337), all these features that are typical to climbing habit is descried as “lianescent vascular syndrome” by Schenck (1893) followed by Carlquist (1985) and Angyalossy *et al.*, (2015). Lahaye *et al.* (2005) studied shrub like habit in the members of the ancestrally lianescent subfamily Secamonoideae of Apocynaceae and reported partially self-supporting growth form *Secamonde sparsifolia* is biomechanically intermediate between self-supporting and lianas. Though, wider vessels have higher efficiency of water conduction (Angyalossy *et al.*, 2011) but they are extremely susceptible to air embolism (Ellmore & Ewers, 1985; Ewers *et al.*, 1991; Rowe & Speck, 1996; Carlquist, 2001; Rajput *et al.*, 2013). Experimental studies carried out on *Tynanthus cognatus* Miers. (Bignoniaceae) by Angyalossy *et al.* (2011) showed that the vessels formed in the centre of the stem remain functional while those located on the periphery of the stem were embolised. Presence of both wider and narrow vessels intermixed with other elements of secondary xylem in plants observed before and after the development of climbing habit may be providing conductive safety.

Formation of the secondary xylem in plates**:** Another interesting feature observed in the climbing habit (including *Cocculus laurifolius*, tree habit) was formation of xylem in radial plates. Formation of such xylem took place due to the presence of relatively wide rays and narrow sector of cambium that produce axial elements. Development of xylem in plates was observed in species with regular secondary growth (*Cissus quadrangularis*, *C. repanda*, *Cissampelos pareira*, *Coscinium fenestratum*, *Stephania japonica*, *Tinosporia cordifolia*, *T. sinensis*, *Vitis vinifera* and *Ephedra foliata* and *E. karumanchiana*) and species with successive cambia (*Anamirta cocculus*, *Cocculus hirsutus*, *C. pendulus*, *Diploclausia glauscence*, *Pachygon ovata* and *Tiliacora racemosa*). Development of secondary xylem in radial plates has been reported earlier in *Aristolochia* (Schenk, 1893; Carlquist, 1988; Fisher & Ewers, 1992; Isnard & Silk, 2009; Rowe *et al.*, 2004) in all the members of the Menispermaceae (Metcalfe & Chalk, 1950; Mennega, 1982; Carlquist, 1996; Rajput & Rao, 2003; Jacques & Franceschi, 2007; Rajput & Gupta, 2015). The occurrence of such wide rays in the members of the Vitaceae has also been reported in previous study (Wheeler & La Pasha, 1994; Rozefelds & Pace, 2018) and Sapindaceae (Chery *et al.,* 2022). These authors correlated wide rays with the increase in stem flexibility as an adaptation to climbing habit (Carlquist, 1985).

***Abundance of parenchyma* (*both axial and ray*)**: Abundance of unlignified parenchyma in wood of scandent plants is attributed as a feature that provides flexibility to the stem and protects large and vulnerable vessels against torsion and damage (Putz & Holbrook, 1991; Carlquist, 1991, 1992 & 2001; Olson, 2003). Xylem of the plants of family Fabaceae and Vitaceae *viz. Canavalia gladiata, C. virosa, Entada rheedii, Phaseolus lunatus, Pueraria tuberosa, Ampelocissus latifolia, Cayratia auriculata, Cayratia trifolia, Cissus quadrangularis, Cissus repanda* and *Tetrastigma bracteolatum* possesses abundant unlignified axial parenchyma whereas libriform fibres were restricted around the vessels or distributed as small islands within the parenchymatous background. Fibres form a sheath around vessels, which is also known as “cable construction”, and has been described in a number of climbing species (Carlquist, 2001).

Abundance of the parenchyma in climbing plants prevents the water conducting tissues during the twisting around host (Putz & Holbrook, 1991). In contrast, Ewers *et al.,* (1991a) showed that how the various wood segments are interconnected in three dimensionally, ensures the continuous supply of water to the vascular system even after damage. The laid-off and interconnected architecture of the vascular system hence provides effective and dependable water delivery to the leaves via a multitude of channels. The parenchyma abundance in xylem and multiple cambial rings also helps to maintain 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 (Schenck, 1893; Haberlandt, 1928; Caballé, 1986; Fisher & Ewers, 1991). Several hypotheses have been developed in relation to the function abundance of parenchyma or the dispersed nature of xylem and phloem units within the parenchymatous background (Carlquist, 1988, 2001a). Few of them are: i) Parenchyma cells enable climbing plants to adjust to changes brought on by the supporting host. These parenchyma cells also allow the stem to be torn while causing the least amount of harm possible to the conducting elements (Schenck, 1893; Carlquist, 1975, 1985, 1988, 2001a). ii) These parenchyma cells offer a location for the storage of starch that can be utilized during a period of intense flowering and fruiting. In some trees, like *Acer saccharum*, the starch in the parenchyma is converted into sugars that are transported into vessels and can be playing important role in increase in osmotic pressure that causes water to flow to those places (Sauter, 1966a, b; Braun, 1970, 1983, 1984; Carlquist, 1985, 1988). iii) The development of new cambia in the parenchyma enables the restoration of the vascular system by substituting functional conducting cells for inactive ones. This hypothesis' offers a level of safety that allows non-damaged xylem and phloem to continue conduction even while some parts of the stem are injured (Dobbins & Fisher, 1986; Fisher & Ewers, 1989; Carlquist, 1988, 2001a). The present study is in agreement with the above said hypothesis since several species in the present study showed formation of interxylary phloem, initiation of the cambium and differentiation of the secondary xylem and phloem in the parenchyma background and sink for the storage of starch.

Formation of successive cambia: Present study revealed development of successive cambia from various cell types like pericycle parenchyma, cortical parenchyma cells, from the dilating phloem parenchyma (both ray and axial) or from both ray and axial the parenchyma cells of the xylem (Metcalfe & Chalk, 1950; Mennega, 1969; Pant & Bhatnagar, 1975; Lowell & Lucansky, 1986; Carlquist & Hanson, 1991; McDonald, 1992; Jacques & Franceschi, 2007; Terrazas *et al.*, 2011; Rajput & Gupta, 2015; Rajput & Gondaliya, 2017; Rajput *et al.*, 2021, 2008, 2014a, b, 2017).

i) *Initiation of successive cambia from pericyclic parenchyma or derivatives*: In *Antigonon leptopus, Cyratia trifoliata, Dicranostyles ampla, Maripa nicaraguensis, Moutabea gentry, Tetrastigma bracteolatum* and *Turbina corymbosa* the increase in stem thickness takes place by forming successive rings of cambia 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. Development of successive cambia in the members of the Convolvulaceae have been reported by earlier researchers (Metcalfe & Chalk, 1950; Mennega, 1969; Pant & Bhatnagar, 1975; Lowell & Lucansky, 1986, 1990; Carlquist & Hanson, 1991; McDonald, 1992; Rajput *et al.*, 2008, 2014a, b, 2017; Terrazas *et al.*, 2011; Rajput & Gondaliya, 2017). Available literature indicates that in many seed plants the pericycle is absent (Evert, 2006) or indistinct. In the present study, in some of the species pericycle was indistinct, in such cases cells located on the inner margin of the pericycle fibres that gave rise to the first cambium are treated as the derivatives of the pericycle (Cabanillas *et al.*, 2017; Moya *et al.*, 2019; Rajput, 2015; Rajput *et al.*, 2008, 2012, 2014; Terrazas *et al.*, 2011).

ii) *Dilatation phloem ray parenchyma and initiation of neo-formed vascular cylinders*: Dilatation of phloem ray parenchyma is a common feature in most of the eudicots (Esau 1969, pp 423). The degree of dilatation is highly variable from species-to-species and is correlated with the adjustment to the increase in diameter in response to secondary growth of stem (Evert, 2007). In this process, ray cells may either extend tangentially or increase tangentially by radial divisions. Extent of radial divisions in rays was observed maximum in some of the investigated species which consequently led to the development of meristem that was structurally and functionally similar to the vascular cambium. In species like *Hewittia malabarica*, *Phaseolus lunatus* and *Turbina corymbosa* the development of neo-formed vascular cylinders were observed from such a dilatation meristem. Most of the species in 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 (Metcalfe & Chalk, 1950; Carlquist & Hanson, 1991; Rajput *et al.*, 2014a, 2021; Lekhak *et al.*, 2018; Kapadane *et al.*, 2019). Unlike other members of the Convolvulaceae, the additional cambia in *Hewittia malabarica* and *Turbina corymbosa* do not connect to form a complete ring of successive cambium and form individual vascular cylinders. Similar vascular cylinders were also observed in *Phaseolus lunatus*, which is reported for the first time in the family Fabaceae. A similar feature known as neo-formed vascular cylinders (Acevedo- odriguez, 2018), is reported earlier in many families of lianas, including the families Bignoniaceae, Euphorbiaceae, Rubiaceae and Sapindaceae (Pace & Angyalossy, 2013; Angyalossy *et al.*, 2015; Bastos *et al.*, 2016; Acevedo-Rodriguez, 2018; Leal *et al.*, 2020; Rajput *et al.*, 2021). Metcalfe and Chalk (1950) referred the term as 'secondary strands of xylem and phloem' to such variant in the genus *Serjania* Miller, while Acevedo-odriguez (2017) termed this as neo-formed vascular cylinders. On the other hand, Angyalossy *et al.*, (2015) defined such additional vascular cylinders formed external to the regular secondary phloem or in cortex as neoformation. Recently Chery *et al.*, (2020) recorded different types of cambial variants exhibited in several species of *Paullinia* L. (Sapindaceae). If compared with these types, cambial variants in *Hewittia malabarica* falls under the lobed xylem and successive cambia types of Chery *et al.*, (2020: p. 279). Samples studied in the present work, these vascular cylinders developed external to the regular phloem and were found to been circled with a ring of the vascular cambium.

Another unique kind of vascular cylinders development was observed in *Turbina corymbosa* and *Argyreia elliptica*. In former species, these cylinders were irregularly oriented and showed presence of xylem and phloem while in later species; they were exclusively composed of xylem fibres and fibriform vessels. With passage of time, several additional cylinders initiated from the parenchymatous conjunctive tissue located between the successive xylem rings (Lekhak *et al.*, 2018; Kapadane *et al.*, 2021).

iii) *Initiation of successive cambia from cortical parenchyma*: Among the species investigated, development of successive cambia from the cortical parenchyma cells were observed in the members of the Menispermaceae, (like *Anamirta cocculus*, *Cocculus hirsutus*, *C. laurifolius*, *C. pendulus*, *Cyclea peltata*, *Diploclisia glaucescens*, *Pachygone ovata* and *Tiliacora racemosa*) and *Rhynchosia pyramidalys* (Fabaceae). There is a difference of opinion regarding the initiation of the successive cambia in Menispermaceae (Maheu, 1902; Obaton, 1960; Rajput & Rao, 2003; Jacques & Fransceschi, 2007; Tamaio *et al.*, 2009; Rajput & Gupta, 2015). According to Maheu (1902), successive cambia in Menispermaceae originate from: i) cortical parenchyma, ii) pericycle, iii) endodermis and iv) irregular activity of the normal vascular cambium (Jacques & Fransceschi, 2007). As shown in figure 21A-C, all the members showing successive cambia, initiate the first successive cambial ring initiate from the cortical parenchyma. This statement is supported with figures showing that the divisions are initiating external to the pericycle fibres. With the initiation of cell divisions, some of the parenchyma differentiates in sclereids while other establish into successive cambium. Further successive cambia develop from the parenchyma cells located external to the sclereids. Similar origin of successive cambia in *Cocculus hirsutus* and *C. laurifolius* has been reported earlier by Rajput and Rao (2003) and Rajput and Gupta (2015) respectively. According to Tamaio *et al.,* (2009) initiation of successive cambia took place from the pericycle by citing the reference of Menezes *et al.,* (2005). This difference of opinion may be associated with the indistinctness of the endodermis and pericycle. Since, the first successive cambium in all the species investigated here initiate from the parenchyma cells few cells external to the pericycle fibres, we consider these cells as cortical parenchyma situated external to the pericyclic fibres. Similar origin of the successive cambia has also been reported in earlier studies (Carlquist, 1996; Rajput & Rao, 2003; Rajput & Gupta, 2015). Carlquist (2007) proposed the term “master cambium” and states that master cambium initiates de novo from the cortical parenchyma (p. 307). However, himself has raised the question that whether master cambium is present in other families that show presence of successive cambia and if yes, then master cambium would be pericycle itself (Tamaio *et al.*, 2009). Since, endodermis and pericycle is indistinct in the species investigated here, further studies are required to confirm the origin of successive cambia.

iv) *Development of functionally inverse cambia*: Development of inverse cambia is a rare feature and is reported in few members (Carlquist & Hanson, 1991, Rajput *et al.*, 2008, 2017; Terrazas *et al.*, 2011; Rajput, 2017; Lekhak *et al.*, 2018; Tamaio *et al.*, 2021; Kapadane *et al.*, 2022) and 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 formed prior to cambium initiation, cells located on the inner margin of meristematic zone differentiate into conjunctive tissue and serve as a site for the initiation of inverse cambia in future, whereas cells on its outer margin becomes the source for the further successive cambia (Rajput *et al.*, 2008, 2014; Terrazas *et al.*, 2011). Terrazas *et al.,* (2011) also reported similar kind of inverted strands of vascular tissue in *Ipomoea arborescens*. Reason for the development of inverse cambia remains unknown and needs further study on this aspect. As per Kim *et al.* (2002), MADS-box genes play a role in parenchyma proliferation in vegetative organs and expression of MADS-box genes (*IbMADS3* and *IbMADS4*) in *Ipomoea batatas* roots not only increase the cell numbers in the vascular cambium but also initiate unusual secondary and tertiary meristems. The present study reveals the proliferation of the ray and axial parenchyma cells was observed in the xylem, indicating possibility of expression of similar genes. According to Spicer and Groover (2010) initiation of inverse cambia is an ontogenetic shift of vascular elements that may be associated with the expression of miRNA resistant Populus REV transgene that shows polarity modification in the secondary vascular tissue. Therefore, it appears that products of inverse cambia may be linked with the translocation and storage of photosynthate to maintain their optimal mobilization in a vertical and horizontal direction (Kapadane *et al.,* 2022). Similar functions have also been ascribed to the derivatives of inverse cambia by Terrazas *et al*., (2011).

***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 the time passed it also showed differentiation of the secondary xylem. Such cambium is referred as ray cambium and is reported earlier by different researchers (Carlquist & Hanson, 1991; Patil *et al.*, 2009; Rajput *et al.*, 2014b; Lekhak *et al.*, 2018). 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. Carlquist (1988) correlated initiation of ray cambium, and formation of phloem from it with the reproductive phase, which is a high energy consuming process.

Therefore, formation of phloem may be connected with quick supply of photosynthate for development of flowers and fruits. As shown in figures 23E, F and 24A, B; proliferation of ray cells also resulted in the splitting of rays that connect the cortex with the pith, which forms an supplementary pathway for the rapid supply of photosynthate to the for developing flowers and fruits. Similar proliferation of rays has also been documented in Malpighiaceae, Bignoniaceae, Cucurbitaceae, Icacinaceae, and Passifloraceae by earlier researchers (Obaton, 1960; Carlquist, 1988; Carlquist, 1992b; Metcalfe & Chalk, 1950; Patil *et al.*, 2011; Cabanillas *et al.*, 2017; Lekhak *et al.*, 2018), referred as fissured stems. Development of vascular elements in the tall and large heterocellular rays is reported by Lev-Yadun & Aloni (1991), and is referred to as 'polycentric rays'. However, rays forming vascular elements in the present study are not polycentric. The development of additional conducting elements (both secondary xylem and phloem) from these ray cambia and proliferation of ray cells may be playing crucial role in rapid translocation of minerals and nutrients, and reserved food materials respectively. However, further studies are warranted to understand this phenomenon of ray and axial parenchyma proliferation. It will also help in understanding its significance in relation to functional and physiological histology of non-self-supporting plants.

Development of interxylary phloem: Interxylary phloem are strands of sieve tubes and associated parenchyma cells embedded within the secondary xylem of the stems and roots and are referred as interxylary phloem or included phloem (Mikesell & Popham, 1976; IAWA Committee,1989; Rajput *et al.*, 2009; Carlquist, 2013). In the present study, development of such phloem 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*. However, this definition broadly includes the phloem formed by successive cambia as well as by a single ring of cambium. Therefore, Carlquist (2001, 2013) advised that not to use this term for plants with successive cambia because in successive rings the secondary phloem lies between secondary xylem and conjunctive tissues. The term interxylary phloem should be used only in case of plants having a single ring of vascular cambium in which the phloem is embedded within the secondary xylem of stems and roots. Histologically and ontogenetically interxylary phloem formation differs from species to species and classified into different subtypes such as Combretum, Strychnos and Azima subtypes (Bonnemain 1969; Philipson 1990) and Calycopteris subtype (Rajput *et al.*, 2009). Development of interxylary phloem in various species of *Strychnos* and *Leptadenia* (including both in trees and lianas) is well documented by earlier workers (Scott & Brebner, 1889; Solereder, 1908; Chalk & Chattaway, 1937; Cockrell, 1941; Singh, 1943; Duvigneaud *et al.,* 1952; Obaton, 1960; Leeuwenberg, 1969; Metcalfe, 1983; van Veenendaal & den Outer, 1993; Araque *et al.*, 2000; Patil & Rajput, 2008; Rajput *et al.,* 2010; Moya *et al.*, 2017). Development of interxylary phloem in *S. bredemeyeri* and *S. andamanesis* fits into Strychnos subtype as described by earlier researchers (Eames & McDaniels, 1947; Metcalfe, 1983; van Veenendaal & den Outer, 1993) and there is no ambiguity about its origin from a single cambium.

Various functions have been ascribed for the presence of interxylary phloem in plants (Rajput, 2003; Carlquist, 2013). *Leptadenia pyrotechnica* is well adapted to xeric condition, where the temperature is very high during sunny days. Available literature indicates that high temperature leads to non-functioning of conducting phloem (Rajput, 2003). Since, the interxylary phloem islands are surrounded by secondary xylem that protects this phloem from high temperature during summer season (Rajput, 2003) and keep the phloem functionally active when the temperature is high during summer. With the passage of time sieve elements of the earlier formed phloem islands become non-conducting and their function is taken over by the newly deposited sieve elements developed from the adjacent parenchyma. Frequent divisions in the parenchyma cells of the phloem islands of both the species of *Strychnos*, *Canavalia* and *Prureria tuberosa* led to initiation of meristematic cells arranged in radial files like cambium. Similar cambial activity has been reported in *Strychnos sp.* (Scott & Brebner, 1889), in *Strychnos bicolor*, by Rajput *et al.*, (2010), in *S. madagascariensis* (Carlquist, 2013), and in *S. bredemeyeri* (Moya *et al.*, 2017). A similar feature has also been observed. In contrast, phloem islands developed with the secondary xylem of both species of *Canavalia* were completely encircled by the vascular cambium. As compared to above species, in *Prureria tuberosa*, the cambium initiated only on one side and it was functionally inverse and deposited only phloem in centripetal direction. According to Carlquist (2013), usually rays do not pass through the phloem islands and if they passing through it, they retain their typical histological characters (i.e., they are lignified). In both species, ray cells passing through the phloem islands are thin-walled, while the same rays were thick walled and lignified when passing through the portion other than phloem/parenchyma islands.

Formation of radially arranged isolated or groups of sieve elements and companion cells in the xylem rays was observed frequently in most of the species. Such development leads to the formation of anastomosing network within interxylary phloem islands and has been reported previously in *Combretum nigricans* and *Strychnos* (Den Outer & van Veenendaal, 1995). Formation of such radially arranged sieve elements in the xylem rays interconnect these islands, which may be associated with rapid translocation of photosynthates. Moreover, their formation in thick-walled xylem rays may protect the phloem from sap feeding insects and may provide insulation for the sieve elements to protect them from dry environment where the temperature is high during summer in plants *L. pyrotechnica*, which is eco-physiologically well adapted to xeric conditions (Migahid *et al.*, 1972).

Development of intraxylary phloem and intraxylary phloem cambium: Internal or intraxylary phloem are strands of phloem differentiating at the pith margin. The occurrence intraxylary phloem is documented in 30 families of the dicotyledons (Metcalfe & Chalk, 1983), including families like Apocynaceae, Convolvulaceae and Loganiaceae. In the present study development of such phloem is documented 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 initiation of primary growth except in *Campsis radicans*. Development of such phloem in this species was observed only after the initiation of secondary growth (Rajput *et al.,* 2018). Available literature indicates that development of primary internal/intraxylary phloem initiates from: i) the procambium (Zózimo *et al.,* 2011), ii) procambial derivatives (Fukuda, 1967; Patil *et al.*, 2009, 2014), iii) immature pith cells, or it may also be secondary in origin, i.e. by dedifferentiation of mature pith cells (Fukuda, 1967; Patil *et al.*, 2014) or by developing the cambium between protoxylem and intraxylary phloem (Fukuda, 1967; Carlquist & Hanson, 1991; Patil & Rajput, 2008; Patil *et al.*, 2009, 2014; Gondaliya & Rajput, 2016; Lekhak *et al.,* 2018; Rajput *et al.,* 2018, 2021, 2022; Tamaio *et al.*, 2021; Kapadane *et al.,* 2022). In the present investigation, all the members of the Convolvulaceae and Loganiaceae initiate from the procambium or procambial derivatives (Rajput & Gondaliya, 2017; Moya *et al*., 2017, 2019; Lekhak *et al.*, 2018; Rajput *et al.*, 2020) while in *Beaumontia jerdomiana* its development was observed from both procambial derivatives and pith cells (Gondaliya *et al.*, 2018). Secondary intraxylary phloem either develops from the mature pith cells or from the internal cambium (also referred as intraxylary phloem cambium) that initiates as small segments between protoxylem and primary intraxylary phloem. Formation of an additional secondary intraxylary phloem was observed by the dedifferentiation of marginal pith cells. A similar way of intraxylary protophloem development is reported by previous researchers in different species of eudicots (Fukuda, 1967; Mikesell & Schroeder, 1984; Lowell & Lucansky, 1986, 1990; Carlquist & Hanson, 1991; McDonald, 1992; Patil & Rajput, 2008; Patil *et al.*, 2009, 2014; Terrazas *et al.*, 2011; Gondaliya & Rajput, 2016; Lekhak *et al.*, 2018; Rajput *et al.*, 2018, 2021, 2022; Tamaio *et al.*, 2021; Kapadane *et al.*, 2022).

Perusal of literature shows that initiation of intraxylary protophloem may occur before, after or concomitant to the formation of regular protophloem (Baranetzky, 1900; Esau, 1939, 1969; Singh, 1943; Fukuda, 1967; Bonnemain, 1972; Kuo & Patel, 1981; Patil & Rajput, 2008, 2014; Patil *et al.*, 2009, 2014; Lekhak *et al.*, 2018; Gondaliya *et al.,*, 2018; Tamaio *et al.*, 2021; Rajput *et al.,* 2022; Ramoliya *et al.*, 2022). In *Beaumontia jerdoniana*, the development of intraxylary protophloem occurs before regular external protophloem. A similar observation has also been documented in *Vallaris solanacea* another member of the same family i.e. Apocynaceae (Gondaliya & Rajput, 2016). The members of Convolvulaceae (like *D. ampla*, *H. malabarica*, *M. nicaraguensis* and *T. corymbosa*) showed simultaneous differentiation of regular and intraxylary phloem. In contrast, *Campsis radicans* showed direct differentiation of secondary intraxylary phloem while protophloem development was found absent.

Information available on the intraxylary phloem also indicates, in thick stem, a segment of cambium initiate between the protoxylem and intraxylary phloem. It is referred as internal/intraxylary/intraxylary phloem cambium/medullary cambium/peri-medullary cambium (Schenck, 1893; Solerder, 1908; Metcalfe & Chalk, 1950; Fukuda, 1967; Pant & Bhatnagar, 1975; Lowell & Lucansky, 1986, 1990; Carlquist & Hanson, 1991; Rajput *et al.,* 2014, Rajput & Gondaliya, 2017; Lekhak *et al.,* 2018; Kapadane *et al.,* 2019; Tamaio *et al.,* 2021). Although the presence of internal cambium is reported in several species, no comprehensive published list is available on genera in which it occurs (Carlquist, 2013). All the species of Apocynaceae, Convolvulaceae and Loganiaceae investigated in the present study showed development of intraxylary phloem cambium. It initiates by the dedifferentiation of parenchymatous cells positioned on the inner margin of the protoxylem and on the outer side of the intraxylary phloem. Our observations on the initiation of this cambium are in agreement with the earlier studies (Schenck, 1893; Solerder, 1908; Metcalfe & Chalk, 1950; Fukuda, 1967; Pant & Bhatnagar, 1975; Lowell & Lucansky, 1986, 1990; Carlquist & Hanson, 1991; Rajput *et al.,* 2008, 2014, 2021; Terrazas *et al.,* 2011; Rajput & Gondaliya, 2017; Lekhak *et al.,* 2018; Kapadane *et al.,* 2019; Tamaio *et al.,* 2021; Ramoliya *et al.,* 2022). Functionally, the internal/intraxylary phloem cambium may be unidirectional and produces only phloem elements or it may become bidirectional in thicker stems and produce xylem centrifugally and phloem centripetally (Fukuda, 1967; Carlquist & Hanson, 1991; Rajput *et al.,* 2008, 2014, 2021; Terrazas *et al.,* 2011; Rajput & Gondaliya, 2016, 2017; Lekhak *et al.,* 2018; Kapadane *et al.,* 2019; Tamaio *et al.,* 2021; Rajput *et al.,* 2022). Most of the species investigated in the present study showed that the intraxylary phloem cambium was initially unidirectional and produced only sieve tube elements. However, with the passage of time, in thick stems, it became bidirectional and showed deposition of both xylem and phloem except both species of *Strychnos* and *Beaumontia* the cambium was found unidirectional even in thick stems available for the study. It appears that formation of intraxylary phloem may be an alternative pathway to provide conductive safety of the photosynthates, i.e. to protect them from external injury by physical damage, insects or herbivores (Gondaliya & Rajput, 2016, 2017; Lekhak *et al.,* 2018; Tamaio *et al.,* 2021; Rajput *et al.,* 2022). Dimensionally, length and diameter of the sieve elements produced by the internal cambium may differ from those of the external normal phloem elements (Patil *et al.*, 2014; Gondaliya & Rajput, 2016; Angyalossy *et al.*, 2015; Rajput & Gondaliya, 2017), and their diameter is reported to be greater in comparable studies (Angyalossy *et al.*, 2015; Gondaliya & Rajput, 2016; Rajput & Gondaliya, 2017). However, studies on dimensional details on the products of intraxylary phloem cambium are meager and further studies on more number of species are needed. The xylem produced from these cambia is more or less similar to the regular xylem and it was composed of vessels (wide and narrow) fibres, axial and ray parenchyma. Among the species studied, *Campsis radicans* showed significant quantity of the xylem production, which crushed the pith portion and completely occupied it (Rajput *et al.,* 2018).

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 (Quintnar-Castillo & Pace, 2022). Evolution of climbing habit induced several alterations in the mechanical architecture of the stem to increase the stem flexibility (Obaton, 1960; Row & Speck, 1996, 1998; Carlquist, 2001; Angyalossy *et al.,* 2015) and growth patterns (Ewer & Fischer, 1989; Carlquist, 2001; Isnard & Silk, 2009; Angyalossy *et al.*, 2015; Lekhak *et al.*, 2018; Rajput *et al.,* 2022). One of them is formation of phloem wedges in which small segments of the vascular cambium either ceases to produce the xylem internally or functionally it becomes slow and increases the phloem production as compared to adjacent portion of the cambium. Such a differential activity of the cambium led to formation of depressions in the xylem, which eventually becomes deep phloem wedges (Rajput *et al.,* 2022). The occurrence of phloem wedges is characteristic to the non-self-supporting habit and is reported in Bignoniaceae (particularly in tribe Bignonieae), Malpighiaceae and Convolvulaceae (De Bary, 1884; Schenck, 1893; Dobbins, 1969, 1971; Metcalfe & Chalk 1983; Fisher & Ewers, 1989, 1992; Carlquist, 2001; Araque *et al.*, 2007; Angyalossy *et al.*, 2015; Pace *et al.*, 2016; Bastose *et al.,* 2016; Kapadane *et al.,* 2021; Quintnar-Castillo & Pace, 2022). In the present study, development of phloem wedges is studied in *Jacquimontia paniculata* (Convolvulaceae), *Serjania Mexicana* (Sapindaceae) and *Turbina corymbosa* (Convolvulaceae). In the young stems (2-4 mm thick stems) of *Arrabidaea candicans* and *Mansoa alliacea* (syn. *Bignonia alliacea*) showing four phloem wedges initially in young stem.

The formation of four phloem wedges in *A. Candicans* and *M. alliacea* followed a similar pattern as described by Dobbins (1969, 1971), Angyalossy *et al.,* (2015) and Pace *et al.,* (2016). Unlike Bignonieae tribe, *S. mexicana* showed no fix number of phloem wedges, rather it formed five wedges first and in subsequent stages of development six more wedges were developed. In *Jacquemontia paniculata* the development of phloem wedges takes place by maintaining a continuous cambial ring and the only difference is that the cambium becomes functionally slow and deposits unlignified parenchyma internally and more phloem externally. With time, these parenchyma cells dedifferentiate into sieve tube elements as reported for other members of the Convolvulaceae (Terrazas *et al.,* 2011; Rajput *et al.,* 2014, 2021; Lekhak *et al.,* 2018; Kapadane *et al.,* 2022). Quintanar-Castilo and Pace (2022) studied development of phloem wedges in Malpighiaceae and reported that phloem wedges develop alternate to the leaf insertion and correlated with the supply of hormones (particularly auxin) and leaf position on the stem. Two different types of ontogenies i.e., i) cambium maintains continuity and ii) interruption of anticlinal divisions in cambium that leads to discontinuation of the cambium have been explained by Quintanar-Castilo and Pace (2022). Similar origin of phloem wedges i.e., alternate to the leaves is also observed in Bignoniaceae by Pace *et al.,* (2009) and

Rajput *et al.*, (2022). In the present study *A. candicans*, *M. alliacea* and *S. mexicana* showed discontinuation of vascular cambium while *Jacquemontia paniculata* showed first type i.e. cambium maintains continuity is observed. Formation of phloem wedges is credited to the shift from self-supporting to non-self-supporting habit to enhance the stem flexibility and protection from damage to the twisting of the stems (Fischer & Ewers, 1989; Carlquist 2001; Rowe *et al.*,, 2004; Isnard & Silk, 2009; Speck & Bugert, 2011).