4. DISCUSSION

Climbing plants differ from trees and shrubs in number of characteristics; most notable is the mechanical properties of the stem (Isnard *et al.* 2003, 2009; Lopes *et al.* 2008). This variation in the mechanical properties of wood is mostly related with a shift from self supporting habit to climbing habit. Lianas have particularly induced several remarkable changes in anatomical and mechanical properties of the secondary xylem, such as: formation of successive cambia; interxylary/included phloem; dissected or compound xylem; furrowed xylem and so on. The shift from self supporting to climbing habit resulted in the development of several interesting structures which differ from the more usual types, thus sometimes referred as anomalous (Cutter 1969) or cambial variant (Carlquist 1988; Sajo and Castro 2006; Tamaio *et al.* 2011). Variant secondary growth is therefore wide spread in lianas and it is considered to increase stem flexibility to protect the phloem, and to increase storage parenchyma. It also limits physical disruption of vascular tissues during twisting and bending, and promotes wound healing after girdling (Dobbins and Fisher 1986; Fisher and Ewers 1992; Lopes *et al.* 2008; Terrazas *et al.* 2011). Major benefit of the variant secondary growth in lianas is the adaptation to climbing habit from the self supporting one.

Although woody climbers (lianas) are very common in tropical forest, anatomical studies regarding them have been very few (Bamber and Ter Welle 1994; Lopes *et al.* 2008; Pace *et al.* 2009, 2011; Lima *et al.* 2010; Tamaio *et al.* 2011; Terrazas *et al.* 2011), and very little attention has been given to the cellular composition of xylem in scandent plants (Carlquist 1985; Araujo and Costa 2006;

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Pace *et al.* 2011; Lima *et al.* 2010; Rajput *et al.* 2010b). However, lianas are the important source of studies regarding the cambial variant, since variant secondary growth is very uncommon in self supporting woody plants (Obaton 1960; Carlquist 1991; Araujo and Costa 2006; Tamaio *et al.* 2011; Terrazas *et al.* 2011). Most of the information on cambial variant is available on the structural details of the climbing plants whereas there are only few studies carried out on the developmental aspect of them (Nair 1993; Patil and Rajput 2008; Rajput *et al.* 2008, 2010b; Lima *et al.* 2010; Pace *et al.* 2011; Tamaio *et al.* 2011; Terrazas *et al.* 2011).

Stems performs enormous variety of roles such as: i) to arrange, orient and support foliage efficiently ii) to overtop competitors and invade new space and iii) to carry water and nutrients to the leaves and sugars and starch to other plant organs (Givnish 1986). In climbing structures including vines and other climbing plants, tensile stress predominate, but the loads are usually supported by the host at more than one point. Consequently such structures even may be more slender than equivalent tensile structures (Givnish 1995). Although it is true that most of the climbing species have slender stem but it is not always true. Sometimes, stems of such species develop various confirmations other than the slender such as lobed (*Dolichos*, *Securidaca*), flattened or winged stems (*Bauhinia surinamensis*), square (some members of *Bignoniaceae*), triangular (*Serjania lethalis, S. laruotiana*) or like a cross as in *Senegalia pedicillata, Phanera outimouta* and so on. These structures are the result of variant secondary growth of the vascular cambium. It may be formed by the vascular cambium normal in position but functionally abnormal in confirmation (Philipson and Ward 1965; Basson and Bierhost 1967; Philipson 1990; Carlquist 2001a).

HABIT:

Since information on many climbing stems has almost exclusively come from xylum material and needs studies on liquid preserved material from field-collected specimens Carlquist (2007a). He has also suggested that improved histological results are less important than habit documentation. For these reasons, we collected specimens and made our observations in the field. It is true that the climbing plants (vines) can be classified as herbaceous vines (sub-woody), as opposed to lianas (woody) that produce thicker stems (Gentry 1991). However, to improve the

characterization of lianas and establish a methodology for working with them, Gerwing *et al.* (2006) offered the following description:. "..........lianas include all climbers that germinate on the forest floor and have true secondary growth (wood) or persistent, fibrous (i.e., "sub-woody" stems)" and "......... herbaceous climbers (i.e., those lacking true wood or persistent, fibrous stems)". Gerwing *et al.* (2006), wrongly state that *Ipomoea* do not have true wood. In fact, since this taxon has secondary growth (Rajput *et al.* 2008; Patil *et al.* 2009); it is easy to understand how confusion can ensue. However, this attempt to distinguish between herbaceous vines and lianas has led to confusions over terminology and interpretations by the absence of clear criteria by which to distinguish; for example, woody from non woody elements or thick from thin stems. Based on conflicting nomenclature in the literature, we are quite aware that the terms herbaceous vines versus lianas is very complex matter, requires further studies on more climbing plants.

INTERNAL/INTRAXYLARY PHLOEM:

Occurrence of internal phloem has been reported in several genera of the dicotyledonous families. Hartig (1854) reported internal phloem for the first time (in Scott and Brebner 1889) when working with *Cucurbita pepo* (Fukuda 1967). Metcalfe and Chalk (1950) listed 27 dicotyledonous families with internal phloem differentiation. However, nearly 95% genera with internal phloem differentiation belong to five orders: Contortales, Cucurbitales, Tubiflorales, Myrtales and Thymeleales and around two-third of the genera with internal phloem belong to the first three orders (Bonneman 1969). Internal phloem also occurs in a number of fern species (Esau 1965). Although, occurrence and distribution pattern of intraxylary phloem is reported in several species, but a search of the available literature did not yield any detailed account of its developmental anatomy of Indian plants except few studies (Singh 1943; Premakumari *et al.* 1985; Patil and Rajput 2008; Patil *et al.* 2009). Internal phloem occur in plant singly or most of the times it is paired with different cambial variants in some plants. As mentioned above in *Leptadenia*, internal as well included phloem observed (Singh 1943; Patil *et al.* 2009), in *I. hederifolia* successive cambia, included phloem, inverse cambia with internal phloem (Rajput *et al.* 2008). In present study development of internal phloem singly or combined with other cambial variants is studied in *Ipomoea hederifolia, I. aquatica, Cressa cretica, Solanum pseudocapsicum* and *Leptadenia reticulata.*

Diversity in the internal phloem development has been reported by earlier workers (Scott and Brebner 1889, 1891; Artschwager 1918; Whitting 1937; Sussex 1955; Philipson and Ward 1965; Fukuda 1967; Mikesell and Schroeder 1984; Patil and Rajput 2008; Patil et al. 2009). According to them, it may develop from the marginal pith parenchyma cells (Singh 1943, Patil and Rajput 2008), the normal procambium (Kennedy and Crafts 1931), procambially derived cells (Fukuda 1967; Mikesell and Schroeder 1984), perimedullary tissue (Worsdell 1915; Artschwager 1918; Woodcock 1935) or from the ground meristem as in tobacco (Esau 1938). Although, several patterns are reported but careful study of the available literature indicates that there is no uniformity in the terminology used to describe the development pattern (Rajput et al. 2010b). Worsdell (1915), Artschwager (1918), and Woodcock (1935) have all referred intervening tissue in which internal phloem differentiates in the younger internodes as perimedullary tissue. On the other hand, Esau (1938) interpreted the cells separating internal phloem strands from rest of the vascular cylinder as ground meristem.

The term proposed by Esau (1938) appears true, because with the advancement of secondary growth in *Ipomoea hederifolia*, *Cressa cretica* and *Solanum pseudacapsicum* the intervening tissue between the protoxylem and internal phloem underwent periclinal divisions and differentiated into an internal cambium. Initially this cambium originated between the primary xylem and primary internal phloem at two opposing areas and later aroused in association with the other areas of internal phloem to form a discontinuous cylinder. In the beginning it was unidirectional and produced only secondary phloem inwardly but later it became bidirectional and produced both secondary phloem and secondary xylem with inverted orientation. Different researchers have reported origin of internal cambium on the outer face of the internal phloem for example Scott and Brebner (1889) in *Strychnos nux-vomica* of the Loganiaceae, Singh (1943) in *Leptadenia spartum*, and Patil and Rajput (2008) in *Leptadenia reticulata* of the Asclepiadaceae. Only phloem was produced internally and there was no outward production of xylem. Lowell and Lucansky (1986) had reported internal cambium in *Ipomoea hederifolia* that was functionally unidirectional, producing only internal phloem. In the present study, we observed same nature of cambium in *Leptadenia reticulata*, *Cressa cretica*, *Solanum pseudocapsicum* and *Strychnos bicolor*. In these species it is uni-directional and

produced only secondary phloem centripetally while in *Ipomoea hederifolia* and *I. aquatica*, it was bi-directional and formed xylem centrifugally and phloem centripetally. Internal cambium was discovered for the first time by Vesque (1875) in Solanaceae, Asclepiadaceae, and Apocynaceae; while Petersen (1882) reported it in Myrtaceae and Lythraceae. Various interpretations have been given by earlier workers for the internal cambium such as: false cambium (Vesque 1875), local cambium (Scott and Brebner 1989), unilateral cambium (Baranetzky 1900), and uni-directional cambium (Philipson and Ward 1965). In the present study, we preferred to use the term "internal cambium" as it is directly concerned with the production of internal phloem. The reason for the development of such inverted orientation of secondary xylem and phloem remained unknown, but this feature appears to be common in *I. hederifolia* (Rajput *et al.* 2008) and also in *I. aquatica*. Presence of internal cambium is reported for the first time in *Solanum pseudocapsicum* and is addition to the list of Solanaceae.

It appears that development of internal phloem from different tissues may be characteristic of a particular group of plants. Differentiation of interxylary/included phloem has been reported in a bicollateral fashion in *Ipomoea versicolor* (Scott 1891), *I. batatas* (Artschwager 1924), *Convolvulus arvensis* (Kennedy and Krafts 1931), in *I. hederifolia* (Lowell and Lucansky 1986), and in *I. quamoclit* (Lowell and Lucansky 1990). In *Ipomoea hederifolia, I. aquatica* and *Cressa cretica* differentiation of internal phloem occurred adjacent to the inner side of the protoxylem, but does not differentiate against the xylem tissue in the younger internodes. Development of such internal phloem indicates that it is not developed from the procambium rather it is formed from the perimedullary cells (i.e. marginal pith cells).

In *Ipomoea hederifolia, I. aquatica* and *Cressa cretica* differentiation of internal phloem occurs adjacent to the inner side of the protoxylem, but does not differentiate against the xylem tissue in the younger internodes. This feature led Herail (1885) and Lamounette (1890) to question the bicollateral nature of such vascular arrangement. To avoid the controversy, the term "bicollateral vascular bundles" was specifically defined by Herail (1885) so that it should be appropriately used only when the internal phloem is derived from the normal procambium at the same time as the normal external phloem. Further, Lamounette (1890) added that internal phloem originates from pith cells and is always developed later in ontogeny

than the normal external phloem. It is evident from Figure 1A and 1B, that internal phloem did not develop from the pith cells but rather, it developed from the procambially derived cells. Fukuda (1967) has also reported that in *Ipomoea pescaprae* internal phloem develops from procambial cells and it all the time originates later than the external phloem.

Internal phloem occurs in the stem of all Solanaceous plants; it lies close to the protoxylem, but generally separated from it by a narrow layer of ground parenchyma. This internal phloem is generally regarded as primary in origin; it arises at the inner margin of the procambial ring by the irregular longitudinal division of certain ground meristem cells to form procambial strands in which the cells differentiate centrifugally into phloem elements (Baranetzky 1900; Esau 1938). However in small number of Solanaceous species, these phloem strands may subsequently become augmented through the cells on their outer edges dividing in a rather orderly manner, with tangential walls predominating, and differentiating into phloem elements centrifugally (Pamela and Wilson 1961). Internal phloem development hitherto been investigated from various angles by many investigators and is still open to controversy. There is no unanimous opinion about the origin of internal phloem.

In present investigation, internal phloem of *Leptadenia reticulata* and *Solanum pseudocapsicum* differentiated from marginal pith parenchyma cells. The tissue separating the vascular cylinder from internal phloem strands has different appearance, and the cells surrounding internal phloem strands are larger in diameter. Additional evidence indicating their origin from the pith cells is evident from Figure 5C, D, that some of the internal phloem strands are quite far from the internal phloem strands located adjacent to the pith margin. Additional internal phloem strands may produce later on by divisions in pith cells. This similar feature also reported in tomato (Venning1949). Differentiation of internal phloem may occur before, after, or simultaneously with the origin of external normal phloem. Internal phloem and normal external phloem arise simultaneously have been reported in several plants such as *Strychnos* and *Apocynum* (Scott and Brebner 1889, 1891), *Cucurbita pepo, Ducesne* (Faber 1904), *Convolvulus* (Kennedy and Crafts 1931), *Cucurbita maxima* (Whitting 1937) and even *Solanum* (Sussex 1955). In the present study, internal phloem arises before the development of normal external phloem. Similar findings also reported, earlier development of internal phloem in Irish potato Artschwager

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(1918), while Mikesell and Schroeder (1984) recorded its origin after the differentiation of external phloem in *Prabhatis nil.*

Interestingly, in the mature stem samples (*Ipomoea hederifolia, I. aquatica, Leptadenia reticulate, Cressa cretica, Solanum pseudocapsicum,* and *Strychnos bicolor*) we noted small cambial arcs (internal cambium) between the inner margin of protoxylem and the outer margin of internal phloem strands; these cambial arcs produced secondary internal phloem towards the center of the pith. Its presence in some though not all the samples supports the observations of Singh (1943) in *Leptadenia.* Scott and Brebner (1891) reported the occurrence of internal cambium in *Periploca* (Asclepiadaceae), and Handa (1936) reported it in *Marsdenia* of the same family. This internal cambium of *Periploca* and *Marsdenia* is said to produce both secondary xylem and secondary phloem. In the present study, *Leptadenia* and *Solanum pseudocapsicum* showed differentiation of secondary phloem while no differentiation of secondary xylem was observed even in the thick stems.

Structure of secondary phloem produced by the internal cambium was more or less similar to that of secondary phloem originated from the external cambia. Although the occurrence of internal cambium was reported by some of the earlier workers (Vesque 1875; Petersen 1882; Worsdell 1915; Scott 1891; Scott and Brebner 1889; Baranetzky 1900; Singh 1943; Philipson *et al.* 1971; Patil and Rajput 2008), there is very little information available on the dimensional details of the xylem and phloem elements differentiated from the internal cambia. In the present investigation development of internal and external phloem contrasted with each other. However, the length of the sieve tube elements differentiating from the internal cambium was greater as compared to the external phloem, while width of the sieve tube elements was more as compared to the external phloem. Variations in the length and width of the sieve tube elements in internal and external phloem may be associated with its source of origin (Patil and Rajput 2008). Similar information is also reported in our earlier study on *Leptadenia.* In the former case, sieve tube elements were directly differentiating from the fusiform cambial cells while in later case they differentiate from parenchyma cells. During the differentiation these cells may be undergoing an increase in length and width, thus resulting in an increase in length and width of the sieve tube elements. Similar observations have also been noticed by Mikesell and Schroeder (1984) in *Pharbitis nil.*

A variety of functional significance has been ascribed to the presence of internal phloem by different workers. Schenck (1893) and most latter workers hypothesized that different anomalous arrangement of secondary tissue including internal phloem greatly increase the mechanical flexibility of liana stem and prevent their breakage when stems are twisted, coiled or bent. Dobbins and Fisher (1986) are of the opinion that the segment of vascular cambium, living phloem, and parenchyma tissue embedded deeply within the secondary xylem provides a reservoir for the regeneration of tissue when the stem is injured. Experimental studies have proven that internal and included phloem helps lianas to maintain apical dominance in girdled stems. It also reduces the chances of disruption of translocated material to the root system and gives an advantage of rapid and vigorous regeneration in wounded stems. The fascicular phloem is restricted to either side of the main xylem in the main vascular bundles, whereas extra-fascicular phloem forms an anastomosing network that interconnects the vascular bundles laterally in the stem and petiole (Turgeon and Oparka 2010; Zhang et al 2010; Patil et al. 2011). Functionally, fascicular phloem is associated with the transport of the products of photosynthesis and other nutrients over long distances from the leaves to sink tissue. In contrast, Golecki et al. (1999) and Zhang et al. (2010) reported that extra-fascicular phloem is associated with the transport of macromolecules, proteins, abundant amino acids and a wide range of unidentified secondary metabolites while it contains very low level of sugars. One of the potential advantages claimed for such conformation is that protection of vessels from damage during torsion in stems of lianas (Schenck 1893; Carlquist 1975; Dobbins and Fisher 1986). This explanation appears valid, because protection from injury is presumably a better option than the recovery from injury. Water storage is another potential function of the large parenchyma zone in a liana stem (Carlquist and Hanson 1991, Rajput et al. 2008; Patil et al. 2011).

MEDULLARY BUNDLES AND CORTICAL BUNDLES:

A large number of families have been reported to possess medullary bundles (Solereder 1908; Metcalfe and Chalk 1950, 1983). Generally these bundles are conjoint, collateral and they are normally oriented i.e. xylem towards centre and phloem towards periphery but they may have inverse orientation also (Davis 1961; Pant and Bhatnagar 1975; Kirchoff et al. 1984). Similarly, in some individuals of *Achyranthes aspera* are reported to have medullary bundles with xylem on two sides

of the phloem like bicollateral vascular bundles (Joshi 1934). In the present investigation, *Argyreia nervosa* showed inverse orientation of medullary bundles, while *Boerhaavia diffusa* and *Mirabilis jalapa* showed normal orientation of medullary bundles as reported by earlier workers (Maheshwari 1930; Pant and Mehra 1961; Pant and Bhatnagar 1975; Kirchoff *et al.* 1984).

Various interpretations have been offered for the presence of medullary bundles, such as: in most cases, the medullary bundles are reported to be a part of the leaf trace systems but *Bougainvillea glabra* and *Phytolacca dioica* are exception to it and they contribute to the vascularization of the buds (Krichoff and Fahn 1984). According to Lambeth (1940) the medullary bundles are cauline in nature in *Apium graveolens*. Therefore, initiation of these bundles may be at any level of the stem and terminate at any level. Frequently, at the nodes, they differentiate so close to the phloem of the normal (regular) bundle that the elements composing them are in contact with the phloem of the normal bundles of a lateral branch. There is no direct connection of the medullary bundles with the leaf traces (Lambeth 1940). According to Davis (1961) these bundles may be parts of the primary vasculature which failed to develop first vascular ring. These bundles might be useful in giving mechanical strength to plant body in initial stage of growth.

The pith in many dicots is a narrow, small tissue that seems to lack a significant physiological role in mature stems (Mauseth 1988b), but many cacti have an extremely broad pith (Bailey 1962, 1963a, b; Mauseth 1989, 1993). In the present study also *Argyreia* showed large pith several medullary bundles distributed irregularly. Based on their absence from other species of Convolvulaceae, medullary bundles appear to be relictually absent in the family and they might have originated during the early stages of the evolution. The presence of medullary bundles should permit *Argyreia* to use broad pith effectively, storing water and starch in it, and then withdrawing them when needed. The pith of many of these cacti is certainly broader than those of most dicots and it constitutes a significant fraction of the stems' volume. Perhaps of particular significance, the pith, being located interior to a thick cortex and wood, is especially well protected from herbivores. The pith is also long-lasting because *Argyreia* do not form any heartwood, and both the pith and all xylem parenchyma cells remain alive as long as the plant remains alive (Mauseth 1988a). The facts that all medullary bundles in *Argyreia* produce phloem continuously

throughout the life of the plant and along with this presence of abundant starch in many pith cells indicate that nutrient transport is an important role. Similar observations are reported in some species of Cacti (Mauseth 1993).

Medullary bundles and broad pith appear to be selectively advantageous in withstanding water stress in the species showing presence of medullary bundles. Mauseth (1993) correlated that in members of the Hylocereeae and the Rhipsalideae have narrow, unvascularized pith, and these plants are restricted to dry sites in wet habitats: the Rhipsalideae consists of the rainforest epiphytes, and the Hylocereeae likewise contains plants that are epiphytic or epilithic in tropical or subtropical wet areas. None occurs naturally in severe deserts as do members of all other tribes. Those genera that have lost medullary bundles have narrow pith and a relatively broad cortex. If similar correlation is established in other members of Convolvulaceae in which medullary bundles are absent also showed that the pith is very narrow. On the contrary, they are present in *Argyreia nervosa*. Presence of medullary bundles *in A. nervosa* may be associated with its habitat as it mostly found growing on the rocks in the natural habitat. Medullary bundles are probably not important in long distance longitudinal transport. At all levels within a stem, the stele has larger, more numerous conducting tissues. If the stele were extensively damaged, the medullary bundles would probably not be sufficient to conduct around the damaged region as in case of cortical bundles which are considered to be insufficient if the stem is severely injured (Mauseth and Sajeva, 1992).

Maheshwari (1930) studied the structure and development of medullary bundles, successive cambia and its derivatives in *Boehaavia diffusa* and compared it with *Mirabilis jalapa*. Our study of mature stem of *Boehaavia diffusa* showed that after cessation of cell division in one cambium, a complete new cambial ring develops in the secondary cortex or else tangentially larger segments of new cambium are formed (Rajput and Rao 1998b), but in *Mirabilis* tangentially small segments are renewed, giving the appearance of interxylary/included phloem. In *Borehaavia diffusa*, conducting elements of secondary xylem and phloem are formed from tangentially wider bands, and conjunctive tissues are formed from tangentially relatively narrow bands of lateral meristem, producing successive alternating concentric rings of xylem and phloem. In *Mirabilis jalapa*, differentiation of xylem and phloem is bidirectional but the rate of cell division towards the phloem side is

very slow. Moreover, development of phloem begins only after the formation of 8-10 xylem derivatives. The number of sieve elements increases slowly and sufficient amount of phloem is accumulated prior to cessation of cambial cell division. Their lower ratio as compared to xylem makes the cambium appear functionally unidirectional. Similar features of xylem and phloem development have been reported in *Pupalia lappacea*, a member of the Amaranthaceae (Rajput and Rao 1999). However, Balfour (1965), and Philipson and Ward (1965) mentioned that in all members of the Amaranthaceae, the Chenopodiaceae and the Nyctaginaceae, secondary thickening meristem is functionally unidirectional, but its low rate of phloem differentiation as compared to xylem is what makes it appear unidirectional (Rajput and Rao 1999). The occurrence of rayless xylem has been reported by earlier workers, and it is restricted to a tiny share of dicotyledons (Gibsson 1978; Carlquist 1988, 2004; Lev-Yadun and Aloni 1995; Rao and Rajput 1998b, 2003; Rajput and Rao 1999). The absence of rays may be total, with rays not formed at all during the entire life span of the plant, or it may be temporary, with raylessness restricted to the juvenile stage (Lev-Yadun and Aloni 1995). In *Mirabilis jalapa*, rays are absent even at senescence. The occurrence of rayless xylem in dicotyledons is frequently observed in species with reduced cambial activity, short fusiform cambial cells, and highly specialised cell types in secondary xylem. These features are all characteristic of *Mirabilis jalapa*. It has been suggested that rayless xylem tends to occur in plant groups in which normal cambial activity was lost during the course of evolution towards an herbaceous mode of structure (Carlquist 1988). The absence of rays in *Mirabilis* may be associated with its herbaceous habit in which the selective value of radially oriented parenchyma is minimal.

Cortical bundles are vascular bundles other than leaf traces found in the cortex of the stems in numerous dicotyledonous families while in certain families (such as the Cactaceae and the Nyctaginaceae) cortical bundles or medullary bundles are present in addition to the normal cylinder of bundles (Eames and McDaniels 1953, Cutter 1969). Metcalfe and Chalk (1950) listed about 37 families showing presence of cortical bundles in the cortex. In the members of Calycanthaceae have similar cortical-bundle systems (Fahn and Bailey 1957), as do certain genera of the Chenopodiaceae (Fahn and Arzee 1959). In some cases, cortical bundles may be present in only a single species of a family, such as *Nyctanthes arbortristis* (Oleaceae)

(Majumdar 1941). Cortical bundles are diagnostically significant because of their taxonomically restricted occurrence. Heinricher (1883) studied development of the cortical bundles in *Centaurea* and correlated them with that of the assimilatory tissues in the stem.

Couroupita also shows presence of cortical bundles in the branches and peduncle. Presence of cortical bundles in the *Couroupita* indicates its mechanical value in supporting the large number of big size fruits on the single peduncle.

Majumdar (1941), studied the anatomy of *Nyctanthes arbortristis* and reported presence of cortical bundles having inverse orientation i.e. xylem towards the periphery and phloem towards the centre. Similar to *Calycanthus*, cortical bundles of *Nyctanthes* are peculiar and they are never directly connected with main axial ring. The marginal bundles run in the petiole with normal orientation of xylem and phloem, which swings round at the node of insertion so as to show inverse orientation in their courses in the axis. Karrfalt (1975) studied nature of cortical bundles in genus *Adenocaulon* (Compositae), and reported stipular origin of the cortical bundles. Therefore, term "accessory cortex" is proposed for the cortex of *Adenocaulon*, where the apparent cortex of the stem consists of the true cortex plus fused portions of stipules (Karrfalt 1975).

In *Couroupita*, development of cortical bundles was similar just like in *Nyctanthes* and *Calycathus* except some variations in their structure and orientation. For example, they may be conjoint and collateral or sometimes only phloem was formed while xylem remained absent. Occasionally, the cambium may develop on both side of the xylem thus it became bicollateral vascular bundle. They may be inversely oriented or may be arranged parallel to the main axis. Initially, they were small, conjoint and collateral or just contained phloem. Structurally, secondary xylem of the cortical bundles was composed of vessel elements, fibres, tracheids, and axial and ray parenchyma cells.

SUCCESSIVE CAMBIA:

During the course of evolution, plants have evolved different modes of secondary thickening for mechanical support and safety of vessels for hydraulic conductivity. From evolutionary point of view it has been accepted that normal cambium is ancestral in dicotyledons and origin of variant types have very likely been

phylogenetic, for they represent alternative solution to the production of secondary tissues (Carlquist 1988; Rajput *et al.* 2008; Patil *et al.* 2011). Among them formation of successive cambia is most common and usually found in climbing species while in trees it is either very rare or almost nil. Although its occurrence is reported in some species (Schenck 1893; Pfeiffer 1926; Chalk and Chattaway 1937; Metcalfe and Chalk 1950; Carlquist 1988; Philipson and Ward 1965; Philipson *et al.*1971; Rajput and Rao 1998b, 1999; Rao and Rajput 1998, 2000; Tamaio *et al.* 2010, 2011; Terrazas *et al.* 2011) they are restricted only to a tiny fraction of dicotyledonous taxa (Carlquist 1988; Rajput *et al.* 2008; Patil *et al.* 2011). In all the reported cases development of successive cambia may vary in their origin from species to species. They usually form secondary xylem centripetally (towards the centre of the stem) and secondary phloem centrifugally (towards the periphery of the stem). In addition to this pattern, present study also reports a completely new pattern in which every alternate ring of successive cambium becomes inversely oriented i.e. secondary xylem centrifugally and secondary phloem centripetally (Rajput *et al.* 2008) in *Ipomoea hederifolia, I. biloba, I. quamoclit,* and *Dolichos lablab.*

The formation of successive cambia has been known for a long time and tends to occur in members of different families. They also occur in stems and roots of familiar plants such as *Amaranthus, Atriplex, Bougainvillea, Chenopodium, Cycas, Mirabilis, Phytolacca* and *Welwitschia.* They occur in the flattened or variously shaped forms of the giant lianoid stems of Phanera (*Bauhinia*), *Gnetum,* and numerous Menispermaceae (Schenck 1893; Pfeiffer 1926; Chalk and Chattaway 1937; Metcalfe and Chalk 1950; Philipson and Ward 1965; Philipson, Ward and Butterfield 1971; Pant and Bhatnagar 1975; Lowell and Lucansky 1986; Carlquist and Hanson 1991; Rajput *et al.* 2008; Tamaio *et al.* 2010, 2011; Terrazas *et al.* 2011). Successive cambia have also been reported in some gymnosperms like *Gnetum* and *Welwitschia* (Carlquist 1996a), *Cycas revoluta* (Handa 1939), *Encephalartos, Lepidozamia* and *Macrozamia* (Stevenson 1990).

However, different workers have interpreted this phenomenon variously with respect to how successive cambia are formed and types of cells produced by them. Product of these cambia is very typical, small segments of cambia produce maximum number of thin walled parenchymatous cells on both centripetal and centrifugal directions while other small segments of it produces conducting elements of xylem

and phloem. This behaviour of cambia in the Aizoaceae, the Chenopodiaceae, the Nyctaginaceae, the Phytolaccaceae, the Amaranthaceae etc., led to the development of different terminology (viz. primary thickening meristem, desmogen, supernumerary cambia, successive cambia etc.) for this meristem. Yet, there is not only controversy about terminology, but also about the origin(s) of the successive cambia and their cambial derivatives as well as about the underlying growth patterns. Different ontogenetic interpretations have led to the distinct terminology (Stevenson and Popham1973, Mikesell and Popham1976; Mikesell 1973, 1979; Yarrow and Popham1981; Carlquist1988, 2004, 2007b; Rajput *et al.* 2009; Tamaio *et al.*2009; Rajput *et al* 2011). As mentioned by Yarrow and Popham (1981), there are at least 65 synonyms to such meristems that are found in the Chenopodiaceae.

Carlquist (2004, 2007a, b) reinvestigated this problem of different terminology by studying eight species from 7 genera of the Nyctaginaceae and 11 genera of the Aizoaceae showing wide range of habits and diverse growth forms. On the basis of his study on root and stem anatomy, he reinterpreted the meristem and proposed a new terminology: "master cambium". In the present investigation we have attempted to verify the characters proposed by Prof. Carlquist (2004, 2007a, b) for master cambium and confirmed these characters in *Spinacia oleracea* from the family Amaranthaceae, which Carlquist (2004, 2007a, b) has hesitantly proposed for the Nyctaginaceae and the Aizoaceae.

Spinacia root increased in diameter by forming successive rings of cambia while in case of *Mirabilis* instead of complete ring larger segments of cambium are renewed outside to the segment that ceased to divide. Each ring/segments of cambium developed from the parenchyma cells outside the phloem produced by previous cambium. All these cambia were functionally bi-directional in which alternate segment of it gave rise to conducting elements of xylem and phloem while rest of the segments exclusively produced conjunctive tissue on both outer and inner side. Earlier workers have also reported similar pattern of cambial origin in other members of the Chenopodiaceae and the Nyctaginaceae. A unique nature of cambium and pattern of product formation in the Chenopodiaceae, the Amaranthaceae and the Nyctaginaceae attracted an attention of several workers (Solereder 1908; Artschwager 1920; Pfeiffer 1926; Ulbrich 1934; Balfour 1965; Philipson and Ward 1965; Studholme and Philipson 1966; Welkie and Coldwell 1970; Philipson *et al.* 1971; Baird and

Blackwell 1980; Rajput and Rao 1999; Carlquist 2007a; Rajput and Patil 2008; Rajput *et al.* 2010a). An attempt has also been made to establish correlation between Chenopodiaceae and other groups of plants that are showing cambial variants (Chalk and Chattaway 1937; Joshi 1937; Metcalfe and Chalk 1950; Philipson *et al.*1971) but no thorough anatomical study has been done on *Spinacia*. Available literature indicates two different views regarding the nature of cambium in the Amaranthaceae, the Chenopodiaceae and the Nyctaginaceae. According to first interpretation, secondary growth is achieved by series of bi-directional successive cambia which produce secondary xylem centripetally and phloem centrifugally (Artschwager 1920, 1926; Metcalfe and Chalk 1950; Esau 1965; Fahn 1975) while second concept is based on a single unidirectional cambium. According to this interpretation it has been considered that in all the members of the Chenopodiaceae, the Nyctaginaceae and the Amaranthaceae cambia are functionally unidirectional (Balfour 1965; Philipson and Ward 1965; Studholme and Philipson 1966).

In the present investigation, bi-directional differentiation of xylem and phloem was observed in *Spinacia*. Similar observations were also reported in *Pupalia lappacea* (Rajput and Rao 1999; Rajput 2001), of the Amaranthaceae and in *Halogeton glomeratus* (Baird and Blackwell 1980), a member of the same family. In the species investigated, the time of xylem and phloem differentiation is not necessarily synchronized thus development of phloem may start simultaneously or after the development of few xylem derivatives. However, the lower production of phloem elements as compared to xylem gives a false impression of a functionally unidirectional cambium (Rajput and Rao 1999). A unidirectional nature of cambium and mode of xylem and phloem development has been described for *Salvadora* (Salvadoraceae) by Singh (1944). It is also reported for *Combretum* (Combretaceae) and *Entrada* of the Leguminosae (Eames and McDaniels 1947). Stem and roots with successive cambia have great adaptive potential. The relative amount of parenchyma, fibres, vessels and sieve tubes may be correlated with its habit. Secondary xylem of *Spinacia* is mainly composed of parenchyma cells in which vessels are the only xylem elements that are having thick wall deposition (lignin deposition).

A type of secondary growth resulting in the production of a higher percentage of parenchyma cells (conjunctive tissue) at the expense of heavily lignified xylem tissue is correlated with its adaptive significance for the water acquisition in many

chenopods (Baird and Blackwell 1980). It may be true in case of these chenopods, as they are considered to be derived from maritime or inland saline areas. In the present investigation it appears that more amount of parenchyma in the secondary xylem may be associated with its habit. *Spinacia* being annual herb with cauline nature do not require more number of thick walled lignified elements for mechanical support. Similar correlation has also been established in the members of closely related families like the Amaranthaceae, the Nyctaginaceae, the Aizoaceae etc. (Joshi 1937; Carlquist 2007a, b; Rajput and Patil 2008). The parenchymatous background of the stem and relatively more parenchyma cells in *Sesuvium* may be associated with its sprawling habit, as it grows on fine sand of the seashore to provide greater flexibility (Rajput and Patil 2008; Rajput *et al.* 2008). Similarly presence of thick, fleshy leaves and thick cortex seems to be associated with water storage because most part of the year plant experience physiological drought (Rajput and Patil 2008). Carlquist (2007b) concluded that habit bears direct relationship to stem and root histology of the Aizoaceae. According to him the highly fibrous stems and roots of *Ruschia* and *Stayneria* correlated with the shrubby structure while fewer and thinner fibrous cylinders of *Mestoklema* and *Aptenia* are related with its sprawling habit. If correlated with habit, formation of successive cambia with abundance of parenchyma may be associated with the storage of reserve food material and modification of roots towards storage habit as seen in case of sugar beet. Thus *Spinacia* may be the beginning towards storage habit and ending with sugar beet. Structural similarity in the vasculature of both the species and occurrence of multiple active cambia at a time is additional evidence towards the increase in more parenchyma for storage of food material.

Secondary thickening in *Antigonon leptopus*, *Diploclisia glaucanscens* and *Cocculus hirsutus* is achieved by the development of successive rings of cambia (Rajput and Rao 2003; Jacques and Franceschi 2007). Carlquist (1996b) showed that the term "successive cambia'□ was more precise than "included phloem'□ (proposed by Metcalfe and Chalk in 1950) to describe this special type of secondary growth. We agree with his analysis because new secondary cambial units appear successively in the pericycle or cortex by the activity of new cambial zones. Tamaio *et al.* (2009) reported that origin of second cambium ring from pericycle but we disagree with her observations as the pericycle and endodermis in *Cocculus* is not that distinct. Another

important point is origin of successive cambia in *Cocculus hirsutus* and *Diploclisia glaucanscens* each new cambium ring originated in cortex 3-4 cells outside to the pericyclic fibre bands that formed a cap above the phloem of vascular bundles.

As reported by Van der Walt *et al.* (1970) in plants with successive cambia; after an active period of cell division, the vascular cambium ceases its activity and a new ring of cambium originates from the cortical parenchyma cells. Our study also supports that, the second ring of cambium in both *Cocculus hirsutus* and *Diploclisia glaucanscens* was developed after the cessation of cell division in previous cambium and new cylinder of cambium was formed from the cortical parenchyma located 3-4 cells outside to the pericyclic fibre bands. These parenchyma cells underwent periclinal divisions forming a wide band of meristematic cells, from which the cells in the middle of the band differentiated into the new cambial ring. The cells outside the newly formed cambium provide the site for the development of the next cambium while the parenchyma cells inside to this new cambium differentiated into conjunctive tissue. Carlquist (1999a, b) coined the term "diffuse lateral meristem" for such cambia because the periclinal divisions do not take place in a single layer as in vascular cambium, but at various points in the radial rows of secondary cortex. In plants with successive cambia, the primary thickening meristem may form by tangential divisions in cortical parenchyma just to the outside of primary vascular tissues. Theoretically third, fourth etc., cambia could develop in a similar way from the cortical parenchyma (Carlquist 2004, 2007b).

The anatomy of vines is the result of selection for specific adaptive structures that will facilitate efficient and safe transport in long and thin stems and recovery from twisting, coiling, crushing, rubbing, and other injuries due to swaying of supports, tree fall, and herbivory (Carlquist 1975, 2001b; Ewers 1985; Fisher and Ewers 1989; Ewers and Fisher 1991; Patil *et al.* 2011; Tamaio *et al.* 2010, 2011; Terrazas *et al.* 2011). One of the anatomical characteristic features of some vines is the formation of huge rays (Carlquist 1975, 1991; Fahn *et al.* 1986; Lev-Yadun and Aloni 1995) which results in segmentation of stem into sectors, which in turn permits stem segments to twist without fracture and to function in regeneration (Carlquist 1975, 1991; Sieber and Kucera 1980; Fisher and Ewers 1989; Lev-Yadun and Aloni 1995; Rajput *et al.* 2010a; Patil *et al.* 2011). Storage and radial transport of reserve materials, water and minerals is considered to be one of the most important functions

of rays (Ziegler 1964; Van Bel 1990; Harms and Sauter 1992; Lev-Yadun and Aloni 1995). Storage of huge amount of starch in rays and fibres and occurrence of large compound rays in *Diploclisia glaucanscens* and *Cocculus hirsutus* may be associated with the storage of starch because of paucity of axial parenchyma.

So far in all the investigated species in which successive cambia have been reported always produce xylem towards the centre and phloem towards the periphery. An unexpected finding of the present study is that in *I. hederifolia*, *I. biloba*, and *I. quamoclit*, every alternating ring of successive cambium is inversely oriented. This cambium produces xylem centrifugally and phloem centripetally. To our knowledge, this type of cambial variant has not been reported previously in any of the species showing successive cambia. While *Dolichos lablab* showed inverse orientation of vascular bundles. However, inverse orientation of vascular bundles has already been reported by earlier workers (Handa 1937; Rajput *et al.* 2006; Rajput *et al.* 2008; Terrazas *et al.* 2011).

INTERXYLARY OR INCLUDED PHLOEM:

Interxylary or included phloem are terms applied to the phloem produced centripetally from the cambium or externally from isolated cambial segments within the secondary xylem (Mikesell and Popham 1976; Carlquist 1988). The term "interxylary phloem" should be restricted to the product of a single cambium produced internally as strands of (interxylary/included) phloem embedded within bands of axial parenchyma surrounded by secondary xylem (Carlquist 2002). The term "included" is a misnomer in the case of dicotyledons with successive cambia, because conjunctive tissue in those species is formed either as background tissue or as bands between one vascular band and another, and thus by definition is not "included" within the secondary xylem of species with successive cambia (Carlquist 2002, 2004).

Depending upon pattern of development, included phloem may take different forms like one or two sieve tubes as in *Canavalia* (Rajput 2003) and little or no phloem as in *Stylidium* (Carlquist 1988), larger bands like *Salvadora* or embedded in confluent axial parenchyma as in *Combretum*, as larger phloem strands tangentially wider than radial width of many Onagraceae (Carlquist 1988) and in *Thumbergia alata* (Carlquist and Zona 1987). The diffuse or foraminate type of phloem development may be categorised in four subtypes: i) *Combretum* subtype, according to Eames and

McDaniels (1947) in genera like *Combretum* small segments of cambium produce phloem cells towards the inside (centripetally) for a brief period, in place of the xylem cells which are normally produced. After a brief period of such activity, these cambium segments restore their normal function and bury the phloem formed inwardly within the xylem. A similar type of interxylary/included phloem development was described in *Salvadora* and other members of the Salvadoraceae (Carlquist 2002), and in *Leptadenia* (Singh 1943; Patil and Rajput 2008). ii) In *Strychnos* subtype xylem production retards over small arcs of the cambium and the original circular outline of the cambium was restored by complementary segment formed outside to it (Eames and McDaniels 1947; Philipson 1990; Van Veenendaal and Den Outer 1993; Den Outer and Van Veenendaal 1995). iii) In *Azima* subtype certain groups of parenchyma cells of the secondary xylem divide (dedifferentiate) and re-differentiate into phloem elements (Philipson 1990; Den Outer and van Veenendaal 1981) and iv) In *Calycopteris* subtype, in small segments of the cambium, cells in the middle of the cambial zone differentiate into their derivatives, thus splitting the cambial zone into outer and inner segments. The outer segment restore cambial cylinder by joining with existing cambial ring while the inner one became encircled by differentiating xylem (Rajput *et al*. 2009).

In *Leptadenia* at places of secondary xylem, cambium form secondary phloem along with thin walled parenchyma cells. This is however, temporary phase and it soon resumes its normal activity producing usual secondary xylem elements. This process is repeated several times giving rise to a number of thin walled tissue embedded in the thick walled cells of the wood. Singh (1943) reported similar pattern of phloem islands formation in *Leptadenia*. He has correlated formation of included phloem of *Leptadenia* with *Strychnos* but in later case origin of included phloem is quite different. In case of *Leptadenia* it originates to the outside and inside to the secondary xylem from the single normal cambium or from the parenchyma cells that yield additional sieve tube elements and companion cells towards inside of the stem. Carlquist (2002) referred residual meristem to such parenchyma that yields included phloem in *Salvadora*. Over a period of time continuous addition of sieve tube elements and companion cells and as the phloem islands are surrounded by thick walled xylem elements there is no scope for expansion of newly formed elements thus results into crushing of older and nonfunctional sieve tube elements.

According to Eames and McDaniels (1947) in genera like *Combretum* small segments of cambium are said to produce phloem cells towards the inside for a brief period, in place of the xylem cells which are normally produced. After a brief period of such activity, these cambium segments restore their normal function and bury the inwardly formed phloem within xylem. A similar description is also offered for the *Combretum* subtype of included phloem development by Philipson (1990). Such type of included phloem development was described for *Combretum nigricans* by Den Outer and Van Veenendaal (1995), in *Salvadora* and other members of the Salvadoraceae (Carlquist 2002) and in *Leptadenia* (Singh 1943; Patil and Rajput 2008). Being a member of the Combretaceae similar pattern of interxylary/included phloem was expected from the *Calycopteris floribunda*. Our observations however, do not match with it. Development of internal phloem in *Calycopteris* not only differs from the *Combretum* subtype but also does not fit into any of the type described so far.

Several studies on development of included phloem have been reported on the Combretaceae (Den Outer and Van Veenendaal 1995; Mikesell and Popham 1976; Mauseth 1988a; Metcalfe and Chalk 1950; Van Vliet 1979), still there is a controversy about the precise sequence of ontogenic events (Metcalfe and Chalk 1983). It is evident from Fig. 44A-D that cambial zone split into outer and inner segment by differentiation of cambial cells located in the middle of cambial zone. Though ontogeny is different but these two segments functionally behave like *Strychnos* type i.e. embedded segments is functionally unidirectional producing only phloem in centrifugal direction. On the other hand outer segment is functionally bidirectional and form xylem towards the centre and phloem towards periphery. In *Strychnos*, subtype xylem production regenerates over small arcs of the cambium and the original circular outline of the cambium was restored by complementary segment formed outside to it (Eames and McDaniels 1947; Philipson 1990; Van Veenendaal and Den Outer 1993; Den Outer and Van Veenendaal 1995).

Photographic evidences (i.e. Fig.1 and 3) provided by Den Outer and Van Veenendaal (1995) are convincing that development of included phloem in the *Combretum nigricans* directly occurs inwardly from the vascular cambium and relatively soon a unidirectional cambial segment is formed in the parenchyma cells at

the inward border of the phloem strand. It is therefore, recommended by them to consider it as diffuse included phloem. On the other hand, Mikesell and Popham (1976) and Carlquist (1988) are of the opinion that since the phloem has been produced by a single cambium, the term interxylary/included phloem can be used instead of diffuse included phloem. According to Mauseth (1988a) this anomalous situation does not exist and the cambium never produces phloem inwardly. Phloem may be formed only as a result of re-differentiation of the xylem parenchyma produced centripetally by the cambium. According to Metcalfe and Chalk (1950) diffuse included phloem occurs in restricted genera of the Combretaceae and is found only in African species investigated (Van Vliet 1979). Furthermore distribution of included phloem is restricted to erect species and absent from lianas except for *Combretum edwardsii* (Den Outer and Van Veenendaal 1995).

The variation of the length and width of sieve tube elements in included and external phloem may be associated with their origin. The dimensions of external sieve tube elements are smaller than in included phloem. External sieve tube elements differentiate directly from fusiform cambial cells, while in included and phloem the fusiform cambial cells differentiate first into parenchyma cells, and later from parenchyma cells into sieve tube elements. During that differentiation from cambial cells to parenchyma cells and from parenchyma cells to sieve tube elements, the cells may undergo an increase in the length and width of the sieve tube elements. Similar observations are reported from a study of internal phloem differentiation in *Ipomoea pharbitis* (Mikesell and Schroder 1984) and in our previous study on *Leptadenia* (Patil and Rajput 2008).

Interxylary/included phloem islands in the roots of *Cucurbita* and *Lagenaria* have been reported by Metcalfe and Chalk (1950). Similarly, Metcalfe and Chalk (1950) reported that in *Thadiantha dubia* interxylary/included phloem arise in the unlignified tissue of the primary medullary rays. In the present study, *Coccinia indica* also showed development of interxylary/included phloem. Formation of interxylary/included phloem by the activity of ray cambium in some members of the Cucurbitaceae has also been reported by Carlquist (1992). In *Coccinia*, in addition to the development of ray cambium, formation of interxylary/included phloem has also found from the axial parenchyma cells. Prior to the development of interxylary/included phloem, axial parenchyma cells became meristematic and

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aligned themselves in radial files like the cambium. From this meristematic zone differentiation of interxylary/included phloem takes place. Various patterns of interxylary/included phloem development appear to be associated with the collapse of parenchyma cells and sieve tube elements. The empty space formed in response to collapse of parenchyma and sieve tube elements seems to be compensated with newly formed other cells.

Zimmermann (1922) and Carlquist (1992) reported three different kinds of cambial variants in different genera of the Cucurbitaceae. These cambial variants may have similar topographic and physiological significance: providing strands of vascular tissues that do not increase the thickness of single woody cylinder but instead form vascular strands separated from the main cylinder (Carlquist 1985, 1991, 1992; Putz and Holbrook 1991). In *Coccinia* also collapse of the parenchyma cells and formation of interxylary/included phloem does not appear to contribute to an increase in thickness of the stem.

In the maturing stems, phloem located on the inner side of the bicollateral vascular bundles (of young stems) undergoes obliteration. This non-functional phloem is replaced by addition of new phloem cells by a cambium-like meristem originated from the adjacent parenchyma cells.

Sieve tube elements which become non-functional usually show massive deposition of callose on sieve plates (Lawton and Lawton 1971; Evert 1984; Deshpande and Rajendrababu 1985; Vishwakarma 1991; Rajput and Rao 1998a). Callose along with P-proteins also plays an important role in the sealing of sieve pores by its deposition in non-functional sieve tube elements but also blocks the exudation of phloem sap in the wounded portions (Oparka and Cruz 2000; Turgeon and Oparka 2010). In our material non-functional sieve elements showed very little accumulation of callose on the sieve pores but as the sieve elements began to obliterate, callose gradually disappeared from the sieve plate. Available information indicates that the extra-fascicular sieve elements are characterized by scanty or absent callose (Turgeon and Oparka 2010). Zhang *et al.* (2010) also reported very low level of P-proteins in the phloem produced by the fascicular cambium. Thus the sealing function may be performed by other major proteins identified in the fascicular phloem. Absence of callose and P-protein from the phloem is hypothesized to release defense compounds

to inhibit pathogens and to protect the sugar rich phloem from insect and fungal attack (Turgeon and Oparka 2010; Zhang *et al.* 2010).

CAMBIUM NORMAL IN FUNCTIONS, ABNORMAL IN CONFORMATION:

A few dicotyledons, including some gymnosperms, have evolved with a pattern that deviates from others; these are known as cambial variants (Carlquist 1988, 2007a; Rajput *et al.* 2008; Kraus and Basconsuelo 2009; Terrazas *et al.* 2011). In such plants, not only the pattern of secondary thickening varies, but sometimes it also changes the outer conformation of the stem. That is, in contrast to the typical slender shape, stems of such species sometimes become lobbed (*Dolichos*, *Securidaca*), flattened or winged (*Bauhinia surinamensis*), square (some members of *Bignoniaceae*), triangular (*Serjania lethalis*, *S. laruotteana*) or cross-like, as in *Senegalia pedicillata*. Such changes may be the product of a single vascular cambium that is normal in position, but functionally abnormal in conformation (Philipson and Ward 1965; Basson and Bierhost 1967; Philipson 1990; Carlquist 2001a).

In *Camptosema isopetalum* and *Phanera glabra* increase in thickness of the stem is achieved by the unequal activity of the cambium on specific side of the stem thus resulting into flattening of the stem. Wagner (1946) also reported similar observations in the *Bauhinia divaricata* and *B. sericella*. It is considered that injuries due to lateral pressure in the conducting tissue, especially in the soft bast, are prevented in climbing plants by the development of ribbon-shaped stems. The one year old stem exhibits normal growth, but during the second year, new cambiums arise at two points near the periphery of the stem. Eames and McDaniels (1953) explain the formation of strap-like stems as due simply to the restriction of the activity of the cambium to certain areas. Schenck (1893) lists three types of anomalous secondary growth as occurring in the genus *Bauhinia*: the formation of a cleft xylem mass; the formation of successively younger zones of xylem and phloem; winging and waving of the stem. The species studied here exhibits only the last mentioned type of cambial variant.

FURROWED XYLEM:

The stem anatomy of species of Bignonieae is quite variable due to the presence of an unusual cambial activity in their stems. It is well known for furrowed xylem or

phloem wedges. According to Dobbins (1969, 1981), formation of number of phloem wedges correlated with phyllotaxy. All reported lianas of the Bignoniceae have a decussate phyllotaxy with one pair of compound leaves located at each node. However, one individual of *Clytostoma callistegoides* possessed a whorled phyllotaxy in two branches. Interestingly, it was found that branches having decussate phyllotaxy showed development of four wedges of phloem while branches with whorled phyllotaxy showed formation of six wedges. In the present study also, *Bignonia alliacea* has opposite decussate phyllotaxy and possess four wedges of phloem. In other members which show multiple phloem wedges in the mature stems do have only four furrows of phloem in the young woody stem initially and additional furrows may be formed later on as the secondary growth progress further (Cruiger 1850; Hovelacque 1888; Schenck 1893; Pfeiffer 1926; Obaton 1960; Dobbins 1971). *Bignonia alliacea* showed formation of only four phloem wedges in whole lifespan of plant.

Formation of four phloem wedges in *Bignonia alliacea* reduces the production of xylem and increase the production of phloem, combined with sieve tubes of wider diameters and greater longevity. The difference in the sieve tube diameters, longevity, and frequency is so different between the variant portions and regular portions that it is very likely that most of the conduction of assimilates may be restricted to the phloem arcs/wedges, similar to what was suggested for the included phloem strands in the stems of *Strychnos millepunctata* (Van Veenendaal and Den Outer 1993). It is well known that although lianas possess narrow stems, their canopies may be as large as those of a tree (Ewers and Fisher 1991), contributing up to 20% of the total biomass of a forest (Perez-Salicrup *et al.* 2001). The xylem of lianas has been shown to be more efficient for water conduction (Ewers 1985; Ewers *et al.* 1990), with some of the widest vessels recorded (upto 500 mm) (Carlquist 1985). Similarly, it is expected that the phloem would exhibit features allowing for a better conduction of photosynthates. In fact, the variant cambia produce wider sieve tube elements not only in the Bignoniaceae, but also in the Hippocrateaceae (Obaton 1960), Loganiaceae (Van Veenendaal and Den Outer 1993), and the Icacinaceae (Lens *et al.* 2008).

Dobbins (1971) reported that in *Doxantha unguiscati* formation of phloem wedges in stem is due to activity of unidirectional cambial arcs while rest of arcs were normally bidirectional. In the initial stages, secondary tissues have a cylindrical configuration, but subsequently four grooves become apparent. These grooves signify

the first evidence of unidirectional cambial activity. The four unidirectional arcs occur near the four major vascular strands to which all of the leaf traces connect. As secondary growth continues, the bidirectional and unidirectional arcs of cambium become separated and radial fissures can be seen between the furrows of phloem and the lobes of secondary xylem. These furrows bordered by multiseriate rays. This is one of the most significant events occurring in the development of the unidirectional arcs is the formation of multiseriate rays that border each furrow. Hovelacque (1888) made a detailed study on the ontogeny of these rays, but did not list any functional significance that might be associated with them. The observations made in the present investigation suggest that the rays may be acting as "physiological barriers". For example, ontogenetically the unidirectional arc is separated from the bidirectional arc at the points where multiseriate rays occur. In this case, the rays may act as barriers that break the homeogenetic induction process and allow the continued development of the unidirectional arcs (Dobbins 1971). Also, if growth substances are involved in regulating unidirectional cambial activity, the rays may act as barriers allowing gradients to be established opposite to certain portions of the cambial cylinder (Dobbins 1971). Similar observations were also found in *Bignonia alliaceae*.

XYLEM IN PLATES:

Aristolochia and *Tinospora* are perennial climbers. In fully grown plants the stem becomes thick, moderately woody, and parenchymatous with very large and wide rays. *Aristolochia* is commonly known for its atypical wood anatomical structure i.e. lignified xylem plates embedded in parenchymatous ray cells. Schenk (1893) named such anomaly as "*Aristolochia*-Typus" first time. Similar structure was also observed in some members of Menispermaceae, for example in genera *Coscinium*, *Borismene*, *Fibraurea*, *Tinomiscium*, *Parabaena*, *Penianthus* and *Tinospora* (Jacques and De Franceschi 2007). Family Menispermaceae is comprised of woody lianas and show cambial variant in almost all members of the family (Tamaio *et al.* 2010). It is well known for having successive cambia but normal secondary growth is also prominently observed in some members (Rajput and Rao 2003; Jacques and De Franceschi 2007; Tamaio *et al.* 2009, 2010). In such members where secondary growth takes place by single ring of vascular cambium always show high and wide rays (Jacques and De Franceschi 2007).

Secondary xylem of *Aristolochia* and *Tinospora* was characterized by the presence of abundant parenchyma. Occurrence of abundant parenchyma in stems of lianas and vine has long been noted (Carlquist 1988; Fisher and Ewers 1992; Lopes *et al.* 2008; Isnard and Silk 2009). Various functions have been ascribed to abundance of parenchyma: they provide greater flexibility (Schenck 1893; Carlquist 1975, 1985, 1988; Rowe *et al.* 2004), site for starch storage (Sauter 1966a, b; Braun 1970, 1983, 1984; Carlquist 1985, 1988), helpful in wound healing process (Dobbins and Fisher 1986; Fisher and Ewers 1989; Carlquist 1988) and also act as background tissue (Carlquist 1988). In stems of these two plants (*Aristolochia* and *Tinospora*), presence of thin-walled parenchyma cells mixed with thick-walled xylem tissues presumably provide higher flexibility to climbing habit (Rowe *et al.* 2004; Isnard and Silk 2009), and may be allowing twisting of stem without damaging the xylem (Carlquist 1988). Alternatively, these structures might also play a role in photosynthate storage (Carlquist 1988) and wound repair (Dobbins and Fisher 1986). Not only abundant axial parenchyma presence is observed in *Aristolochia* and *Tinospora* but both the species also showed non-lignified, very high and wide rays. The two types of ray histology coexist in Menispermaceae and many species of climbers of other families (Tamaio *et al.* 2010). According to the works of Mennega (1982), Carlquist (1996b) and Tamaio *et al.* (2009, 2010), the lignified rays are more common in the plant species showing successive cambia.

Wide and long vessels are common in lianas, but they do possess narrow vessels referred as fibriform vessels. One of interesting character showed by the plant studied in the present investigation is the presence of vessel dimorphism. Vessel dimorphism is a term that can be applied to the presence of wide plus narrow vessel elements (Carlquist 1981; Carlquist and Hanson 1991). Narrow vessel elements are considered to be at least as effective as tracheids in resisting the formation of air embolism in vessels, because air embolism forms far less commonly in narrow vessels than in wide ones (Ellmore and Ewers 1985; Carlquist and Hanson 1991). Thus, Narrow vessel elements, which are common in *Aristolochia*, *Tinspora* and other lianas, can form a subsidiary conductive system with a degree of conductive safety virtually as high as that provided by tracheids (Ayensu and Stern 1964; Carlquist 1991).

COMBINATIONS OF CAMBIAL VARIANT:

Sometimes, plants with cambial variant share more than two different types of unusual secondary growth and it is difficult to segregate them in a specific type of cambial variants, such pattern of secondary growth is referred as combination of cambial variants (Carlquist 1988; Patil *et al.* 2011). Some examples encountered during this study do not confirm to any single type. For example, flattened stems can be found in lianas with successive cambia. Flattened stem is an example of cambia normal in function but abnormal in function but such flattened stems often show development of successive cambia. Similarly *I. triloba* showed lobed stem with successive cambia, *Cocculus hirsutus,* shows flattened stem with successive cambia. *Cocculus hirsutus* member of family Menispermaceae and this family are comprised almost entirely of lianas (Jacques and De Franceschi 2007; Tamaio *et al.* 2010). Study of its wood anatomy is of interest for understanding adaptation to the liana habit. As is true for most liana wood, Menispermaceae wood has not been studied often. However, previous studies showed that it exhibits some interesting features (Santos 1928, 1931; Mennega 1982; Carlquist 1996b): successive cambia occur in most Menispermaceae. Carlquist (1996b) even proposed that successive cambia could be the only secondary growth type in Menispermaceae. Secondary growth in *Cocculus hirsutus* is achieved by the development of successive rings of cambia (Rajput and Rao 2003; Jacques and De Franceschi 2007) and we also came across the same observation. Development of successive cambia from the pericyclic parenchyma have been reported in several other species including the members of the Menispemaceae (Tamaio *et al.* 2009; Jacques and De Franceschi 2007) and they are frequent in Caryophyllales (Carlquist 2001a). As reported by Van der Walt *et al.* (1970), after an active period the vascular cambium ceases its activity and a new ring of cambium originates from the cortical parenchyma cells. Our study also indicates that the second ring of cambium develops from the cortical parenchyma adjacent to the pericyclic fibre band. Occurrence of eccentric successive cambia has been reported in the members of the Menispermaceae, these eccentric cambia in the Menispermaceae result into flattened stem and these cambia are always found to develop only on one side (Rajput and Rao 2003; Jacques and De Franceschi 2007) similar observation were observed in the present investigation eccentric successive cambia are developed on one side of the stem.

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Coccinia indica is a perennial climber with bicollateral vascular bundles. In fully grown plants the stem becomes thick, moderately woody, and parenchymatous with very large and wide rays in almost all the genera of the Cucurbitaceae (Carlquist 1992; Olson 2003; Patil *et al.* 2011). A single vascular cambium is responsible for the secondary growth throughout the life of the plants. However, *Dendrosicyos socotrana* is an exception in having successive cambia in the Cucurbitales (Olson 2003). In *Dendrosicyos*, each xylem strand bears vascular cambium that produces xylem and phloem like other dicots with successive cambia. Presence of successive cambia in this genus may be associated with its habit i.e. it is an example of pachycaul/bottle tree habit. Although, *Coccinia* is a perennial climber, it has most of the features like very wide rays, minimal libriform fibres, and wider vessels similar to that of *Dendrosicyos*. Olson (2003) correlated all the above features with its habit but it appears to be the features characteristic to Cucurbitaceae. Carlquist (1992) reported similar features in other woody genera from the same family. The various features of the wood of the *Coccinia* are related directly or indirectly to the scandent habit. Presence of wide vessels in climbing plants is considered to compensate for the small transactional area of secondary xylem (Carlquist 1992). Cucurbitaceae is known for the greater diameter than the length of the vessel elements. In the present study also average vessel diameter is almost 1.5 times more than its length.

Abundance of parenchyma in wood of scandent plants is cited as a feature that provides flexibility to the stem and protects large and vulnerable vessels against torsion (Carlquist 1991, 1992; Putz and Holbrook 1991). As seen in transverse view, xylem of *C. indica* possesses libriform fibres, arranged around the vessels forming like aliform arrangement similar to that of axial parenchyma cells that form in other dicots. Therefore, the strands of secondary xylem are arranged in the background of soft parenchyma and the vessels are sheathed with libriform fibres. Presence of fibre dimorphism in *C. indica* is not a new feature and is reported in the wood of other species of the Cucurbitaceae by earlier workers (Olson 2003). These fibres initially appear like non-septate fibres but careful observation of these fibres reveals that non-septate fibres are relatively short, relatively thin walled with more lumen diameter. Pits on the radial walls are also circular and relatively larger in size as compared to slit like pits of the septate fibres. Olson (2003) has also reported fibre dimorphism i.e. septate and non-septate fibres in *Dendrosicyos*. As per Olson's report the large, pitted

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parenchyma like fibres are usually non-septate and form the bulk of tissue in *Dendrosicyos* stem. However, our observation on fibre dimorphism coincides with the description given by Olson (2003) while we did not notice any parenchyma dimorphism in *Coccinia* in the present study and do not confirm the observations as described by Carlquist (1992) in other members of the Cucurbitceae. It appears that the non-septate fibres reported by Olson (2003) in *Dendrosicyos* and in the present study may be the transition between axial parenchyma and fibres.

Occurrence of parenchyma dimorphism in other members of Cucurbitaceae is correlated with the scandent habit (Carlquist 1992). The lignified thick walled parenchyma may prevent damage to wide vessel by enhancing the mechanical strength while thin walled parenchyma may offer enhanced flexibility to the stem when the torsion occurs. One of the potential advantages claimed for such a conformation is the protection of vessels from damage during torsion in stems of lianas (Schenck 1893; Carlquist 1975). This explanation appears to be valid, because protection from injury is presumably a better option than recovery from injury. Moreover, thin walled parenchyma cells may be playing important role in the storage of reserve food material as well as in storage of water. Usually *Coccinia* grows in scrub forest and it begins to sprout new leaves during the dryer part of the year before the arrival of rains in the month of June. Thus, abundance of axial parenchyma in the stem of *Coccinia* may be helpful to store enough water as well as reserve food material to produce new crop of leaves during the drier part of the year prior to arrival of rains.

Secondary growth in *Securidaca rivinaefolia* is achieved by the formation of eccentric successive rings of cambia. Formation of successive cambial rings is typical and appears to be characteristic feature of *Securidaca rivinaefolia* that results into dumbbell shaped emblematic stem morphology. Initial 2-3 cambia form complete ring but there after they become eccentric and half moon shaped thus giving characteristic stem morphology. The first successive cambium was developed from the pericyclic parenchyma outside to the phloem produced by previous cambial ring. Development of successive cambia from the pericyclic parenchyma have been reported in several other species including the members of the Menispemaceae (Tamaio *et al.* 2009; Jacques and De Franceschi 2007) and they are frequent in Caryophyllales (Carlquist 2001a). Occurrence of eccentric successive cambia has been reported in the members

of the Menispermaceae, these eccentric cambia in the Menispermaceae result into flattened stem and these cambia are always found to develop only on one side (Rajput and Rao 2003; Jacques and De Franceschi 2007) but in the present investigation eccentric successive cambia are developed on either side of the stem thus leading to dumbbell shape of the stem in all the samples investigated. The Polygalaceae is considered being closely associated with the Fabaceae, Surianaceae and Quillajaceae (APG web site updated July 2009). However, the Fabaceae is well known for its cambial variant starting from very simple like diffuse interxylary/included phloem (Metcalfe and Chalk 1983; Rajput 2003) to complex ones like the *Dolichos* (Rajput *et al.* 2006). In most of members of the Fabaceae xylem is embedded in parenchymatous back ground but in case of *Securidaca* thin walled parenchyma cells are very little and wood is hard. To some extent, stem anatomy of *Rhynchonsia phaseoloides* of Fabaceae and *Securidaca* is similar. *Rhynchonsia* is well known for its flat ribbon like structure due to formation of successive cambia (Schenk 1893). Both the species, *Rhynchonsia phaseoloides* of Fabaceae and *Securidaca* form eccentric and half moon shaped successive cambia on opposite lateral sides of the stem that results in the flattening of the stem. The stem becomes flat thus, on the basis of single species; it is difficult to establish an anatomical correlation between the Fabaceae and the Polygalaceae, moreover one need to study more number of genera from the Polygalaceae to establish the correlation with the Fabaceae.

Prior to the formation of new ring of the cambium, the pericyclic parenchyma cells undergo repeated periclinal divisions and formed 3-4 cell wide parenchymatous band arranged in radial file. From these cells differentiated secondary xylem and phloem, cells on the inner side differentiate into conjunctive tissue, and outer cells act as a site for the future cambium. This pattern of origin of new cambium is similar to different members of the Caryophyllales studied so far (Carlquist 2003, 2004, 2007b; Rajput 2001, 2003). In the Caryophyllales, the band of parenchyma cells that differentiate into new cambial ring is distinct and multi layered but in *Securidaca* before the development of cambial segment the cells start differentiating into secondary xylem and phloem. Therefore, a distinct cambial zone with radially arranged cells may be seen only after the differentiation of 10-15 xylem derivatives. Interestingly first two successive cambia formed complete cylinder but there after each successive ring start on opposite side to each other and spreads bi-directionally

and before they join each other they get fused with previous ring. Such behaviour of the cambium results the stem become morphologically lobbed and dumbbell shaped.

The stem and wood anatomy of Convolvulaceae has been studied by different workers, with the most important contributions being those of D'Almeida and Patil (1945, 1946), Metcalfe and Chalk (1950), Mennega (1969), Pant and Bhatnagar (1975), McDonald (1981, 1992), Lowell and Lucansky (1986), and Carlquist and Hanson (1991). These studies have shown that most of the genera form successive cambia, and that intraxylary phloem is a characteristic feature of the family. Similarly *I. triloba* showed lobed stem with successive cambia, intraxylary phloem and internal cambium.

Morphologically, in *I. hederifolia*, the stem portion adjacent to ground level and not in contact with any supporting object remains circular in outline and shows distinct concentric rings of cambia. However, the portion away from the ground and in contact with the supporting object shows differential cambial activity, ultimately resulting in a lobed pattern. The number of lobes is not constant and may vary for a given plant. The stem is often deeply furrowed, flattened, broadly lobed, or cylindrical. The change in the shape of the stem may be correlated with cambial variants and an unequal production of secondary vascular tissues in the stem (Rajput *et al.* 2008). Similarly in *I. triloba*, young stem showed circular to finely lobed stem, as secondary growth progressed in matured stem it becomes prominently tri-lobed.

In *I. triloba*, the first ring of cambium remains functional for a definite period and later ceases to divide. A new ring of cambium is developed from the axial parenchyma cells at a distance of about three to six cell layers outside the phloem produced by the previous cambium. D'Almeida and Patil (1945, 1946) and Pant and Bhatnagar (1975) have reported similar observations in other species of *Ipomoea* and *Argyreia*. However, the development of successive cambia of many plant families generally arises external to the areas of specialized vascular growth of normally positioned cambium (Studholme and Philipson 1966; Esau and Cheadle 1969; Lowell and Lucansky 1986, 1990; Rajput and Rao 1999, 2000, 2002; Rajput 2001, 2003).

An internal cambium arises in the mature stems of *I. triloba* from parenchyma cells between the primary xylem and internal phloem. It produces secondary phloem centripetally and secondary xylem centrifugally. The development of internal phloem

remains characteristic to some families of dicotyledons, with Convolvulaceae being one of them. This internal cambium is functionally bidirectional, producing secondary xylem centrifugally and secondary phloem centripetally. The formation of such internal cambium, similar in origin, position, and product, also arises in the pith region of *I. versicolor* (Scott 1891), *Calystegia* (Philipson *et al.*1971), *I. quamoclit* (Lowell and Lucansky 1990), and *Ericybe coccinea, Operculina palmerie*, and *Stictocardia benaviensis*, members of the same family (Carlquist and Hanson 1991).

XYLEM STRUCTURE:

Tyloses: Metcalfe and Chalk (1983) offered a listing of families, in which tyloses have been reported. Tyloses are reported in several plant families, but the ability to form tyloses with thick lignified walls is a characteristic feature of few taxa only. Such sclerosed tyloses have been reported in Asteraceae, Scytopetalaceae (Carlquist 1988), Connaraceae (Dickison 1972), Lauraceae (Stern 1954), Myrtaceae (Foster 1967) and in Menispermaceae (Rajput and Rao 2003) present in genus *Cocculus hirsutus*. Sclerosed pitted tylosis were oserved in *Diploclisia glaucanscens* while in *Cocculus hirsutus* simple tyloses observed.

Radial Vascular Elements: Rays are the radial sheets of parenchyma but other elements such as radial tracheids (Fahn 1990), vessels (Carlquist 1988), resin ducts (Fahn 1990), and sieve tubes (Lev-Yadun and Aloni 1991; Rajput and Rao 1998a) can also be found in various species. However, occurrence of such radial vascular elements is a rare feature and they are more common in conifers, many of which have radial tracheids (Carlquist 1988; Fahn 1990; Lev-Yadun and Aloni 1991; Rajput and Rao 1998b). Occurrence of radial phloem strands are reported in *Azima tetracantha* (Den Outer and Van Veenendaal 1981), in *Suaeda monoica* (Lev-Yadun and Aloni 1991) and isolated sieve tube elements in *Tectona grandis, Acacia nilotica, Azadirachta indica, Guazuma tomentosa* and *Erythrina indica* (Rajput and Rao 1998a). Radial fibres are very infrequent and they are reported in the rays of *Monopteryx uacu* from the family Papilionaceae (Metcalfe and Chalk 1950) and libriform fibres in *Cecropia* (Carlquist 1988). In the present study also occurrence of radially oriented fibres in the rays is observed in both, *Cocculus hirsutus* and *Diploclisia glaucanscens*.

The length of the vessel elements and xylem fibres increases from pith to periphery (Rajput and Rao 2003) and similar feature is also noticed in *Diploclisia glaucanscens*. It is more like the paedomorphic curve than the length-on-age curve for woody dicotyledons that have a single cambium, in which marked increase in vessel element length occurs with the onset of secondary growth (Bailey and Tupper 1918). However, Horak (1981) recorded a decrease in the vessel element length for each of seven successive secondary xylem increments in *Stegnosperma* stems. According to Carlquist (1999a), if each of the cambia in a species with successive cambia originates in the secondary cortex, then its products should be similar in length. In *Cocculus hirsutus* and *Diploclisia glaucanscens*, each of the cambia produces xylem for two to three years, so that marked changes in vessel elements and fibre length over time are expected. As in woody dicotyledons with a single cambium, the cambium can persist throughout the life span of the plant and thus changes in length of xylem derivatives can occur over time due to juvenilism.

In the present study vessel lumen diameter is correlated with its frequency and it shows positive correlation. Although, it has been suggested that the number of vessels per mm^2 might easily be regarded as an inverse of vessel diameter but that would be a mistaken and the relationship is by no means perfectly inverse (Carlquist 1988). However, a wide vessel diameter contributes to greater conductive efficiency (Carlquist 1975), narrow vessels tend to develop embolism much less readily than wide ones (Zimmermann and Brown 1971; Ewers 1985), wider vessels are less capable of recovery from embolism than narrow ones (Ewers 1985).

RAYLESS NATURE OF XYLEM:

Raylessness tends to occur in plants with limited cambial activity and results in the scanty accumulation of secondary xylem (Carlquist 1970). If a single cambium can no longer produce more xylem, then successive cambia may increase stem or root diameter. Such a xylem always remains rayless has been reported earlier by Barghoorn (1941) and Carlquist (1970). However, in the present investigation *Spinacia* show a scanty accumulation of secondary xylem by forming of successive cambia. Absence of rays is not a common feature and is restricted to a few species of dicotyledons belonging to quite different families (Carlquist 1988; Lev-Yadun and

Aloni 1995; Rao and Rajput 1998; Rajput and Rao 1998b, 1999). Here with we add *Spinacia* to the list of rayless xylem, which has not been reported earlier.

Absence of rays is not a common feature and is restricted to a few species of dicotyledons belonging to quite different families (Carlquist 1988; Lev-Yadun and Aloni 1995; Rajput and Rao 1998b, 1999; Rao and Rajput 1998). We add here a few more species belonging to the family Amaranthaceae to the list of rayless xylem, which has not been reported earlier. However, rays if produced in species with principally rayless xylem occur only in the outer portion of the secondary xylem, as noted by Barghoorn (1941) for *Geranium tridens*, for *Plantago webbii* by Carlquist (1988) and for *Suaeda monoica* by Lev-Yadun and Aloni (1991).

Ray initials were eliminated from the cambium of *Glycine max* following 2, 4-DB application (Pizzolato 1982). Thus, changes in hormonal stimulation are probably involved in the transition from a juvenile rayless stage to a mature wood containing rays in plants, which display juvenile raylessness (Lev-Yadun and Aloni 1991). Lev-Yadun and Aloni (1991) suggested that this aspect of ray initiation and development is a specific case of a more general phenomenon of the gradual initiation and increase in ray size following maturation of wood in plants. This general pattern of an increase in the size of rays, vessels, tracheids and fibres is correlated with age and distance from the stem apex of the plant (Aloni and Zimmermann 1983; Saks and Aloni 1985; Bhat *et al.*, 1989; Lev-Yadun and Alon 1991). This gradient in the dimensions in the vascular elements and fibres have been explained by a decreasing gradient of auxin concentration from leaves to roots (Aloni and Zimmermann 1983; Aloni 1987, 1991). It has been proposed that the regulation of ray size is influenced by a decrease in auxin level from leaves to roots (Lev-Yadun and Aloni 1991). The decrease in axial polar auxin flow from leaves to roots results in a relative increase in the effect of radial signal flow, which may be responsible for the development of rays in temporary rayless plants.

One of the evolutionary trends found in cambial activity is to result in herbaceous plants types. When secondary woodiness develops the fusiform cambial cells are very short and give rise to highly specialized cell types in the secondary xylem (Gibson 1978). Similar trends were found in all species used in the present study.

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