3. RESULTS

Development of unusual patterns of growth in the present study is broadly categorized into: I) Unusual Primary and II) Unusual Secondary growth.

I. Unusual Primary Growth

3.1 INTERNAL PHLOEM

Phloem which is formed at the margin between pith and primary xylem is termed as internal phloem or intraxylary phloem. However, it is easy to be confused with "interxylary phloem". Therefore to avoid this confusion of terminology, the term *internal phloem* is used for the same. Internal phloem is arranged as discrete strands, and it develops from the marginal semi-mature parenchyma cells of the pith (Fukuda 1967; Mikesell and Schroeder 1984; Grignon *et al.* 1992; Patil and Rajput 2008). This internal phloem is generally regarded as primary in origin; it arises at the inner margin of the procambial cells by the irregular longitudinal division of certain ground meristem cells to form a procambial strands, from which some of the cells differentiate centrifugally into phloem elements (Baranetzky 1900; Esau 1938; Fukuda 1967; Mikesell and Schroeder 1984; Grignon *et al.* 1992; Patil and Rajput 2008). Thereafter, protoxylem elements are differentiated from the procambium between the internal and external protophloem. In transverse view, external phloem differentiates as a continuous band over a period of time. In mature stems, internal protophloem becomes non-functional owing to development of additional new phloem strands and heavy accumulation of callose. The older and non-functional

sieve tube elements of the earlier formed internal phloem strands are replaced by the addition of new phloem strands by the marginal pith cells.

As the secondary growth progress further, sometime in mature stems the marginal pith cells acquired meristematic characters and differentiate into internal cambium (Kennedy and Crafts 1931; Hayward 1938; Mikesell and Schroeder 1984; Patil and Rajput 2008). Internal cambium was reported for the first time in the Solanaceae, the Asclepiadaceae and the Apocynaceae. Though intraxylary phloem is reported in several other families, there are very few species that develop internal cambium at the margin of pith (Fukuda 1967; Mikesell and Schroeder 1984; Patil and Rajput 2008; Patil *et al.* 2009). Differentiation of internal phloem is not synchronised, internal phloem may develop before, after or simultaneously with the development of external normal phloem (Scott and Brebner 1889, 1891; Artschwager 1918; Whitting 1937; Sussex 1955; Fukuda 1967; Mikesell and Schroeder 1984; Patil and Rajput 2008; Patil *et al.* 2009). Although internal phloem is known to occur in a number of dicotyledonous families, it is comparatively rare and restricted to a tiny portion of dicotyledonous families (Philipson and Ward 1965; Carlquist 1988; 2001; Patil *et al.* 2009). Metcalfe and Chalk (1983) reported about 26 families showing presence of internal phloem.

As mentioned earlier that sometimes a cambium originates between the primary xylem and internal phloem, called as internal cambium and is reported by earlier workers also (Fukuda 1967; Mikesell and Schroeder 1984; Patil and Rajput 2008; Patil *et al.* 2009). In the present study, however all the plants studied not only showed development of internal cambium but some members also showed development of secondary xylem and phloem from the internal cambium while other members showed only development of intraxylary/internal secondary phloem. Therefore, some portion of secondary growth is also included in this section although having title "Unusual Primary Growth" along with the development of internal phloem, present investigation also gathers additional information on origin and development of internal cambium and production of intraxylary secondary phloem and secondary xylem. Structure of internal secondary xylem and internal secondary phloem is compared with the secondary xylem and phloem formed by external cambium. Following plants are studied to investigate structure and development of internal phloem, development of internal cambium and its derivatives: *Ipomoea*

hederifolia, *I. aquatica*, *Leptadenia reticulata*, *Cressa cretica*, *Solanum pseudocapsicum*, and *Strychnos bicolour*.

3.1.1 *Ipomoea hederifolia* L. and *Ipomoea aquatica* Forssk.

***Ipomoea hederifolia* L.** (Convolvulaceae)
Common Names: *Red Star Glory, Yellow Ivy Leaf Morning Glory and Yellow Trumpet Morning Glory*

***Ipomoea aquatica* Forssk.** (Convolvulaceae)
Common Names: *Water-spinach, Chinese water-spinach or morning-glory* and *water bindweed.*

Anatomy of young stems: In the young stems of both *Ipomoea hederifolia* and *I. aquatica*, the epidermis was composed of single layered, compactly arranged isodiametric parenchyma cells. It was covered with a thin cuticle on the outer side while internally it contained 2-3 layered hypodermis comprising thin walled isodiametric parenchyma cells. The cortex was mainly composed of parenchyma cells along with several immature laticifers that were distributed randomly in it. In *I. hederifolia*, they were also developed in the pith simultaneously with the differentiation of internal phloem strands (Fig. 1A, B). On the other hand in *I. aquatica*, comparatively more laticifers were found in cortex region (Fig. 4A, B), while central portion of the pith region was distorted from the very young age of the stem. In both the species, formation of internal phloem may be divided into two distinct types: i) from the procambially derived cells and ii) from the internal cambium.

Development of internal phloem: In both the species, differentiation of primary external protophloem precedes that of internal protophloem. Isolated strands of internal protophloem began to differentiate only after the formation of procambial initials (Figs. 1A, B, 4A, B). The procambium was frequently interrupted by alternate bands of parenchyma cells. At this stage, the stem was composed of several vascular bundles comprised of external protophloem, protoxylem and strands of few internal protophloem elements in the third internode (Figs. 1C, 4B, C). Subsequently tangential divisions occurred in the interfascicular region that resulted in the

development of a complete cylinder of the cambium like meristem (i.e. metacambium) in the sixth internode. Periclinal divisions in this meristem differentiated metaxylem centripetally and metaphloem centrifugally (Figs. 1D, E, 4C, D). The first formed metaxylem vessel members were relatively narrow and possessed helical or reticulate thickening. Metaphloem was composed of relatively narrow sieve tubes, companion cells and phloem parenchyma cells. At this stage, patches of internal phloem were differentiated from the procambially derived cells. These newly formed phloem elements were located between first formed protoxylem elements and internal protophloem. Some of the parenchyma cells divided subsequently and produced a group of relatively small cells as compared to adjacent pith cells (Figs. 1F, 4D) and served as a site for the origin of the internal cambium in future. By this time complete ring on cambium was developed on the external side producing normal secondary phloem externally and secondary xylem internally. Prior to origin of the internal cambium, the cells located between the protoxylem and internal phloem formed additional internal secondary phloem by de-differentiation and re-differentiation (Fig. 1F). Considerable amount of internal phloem was accumulated prior to development of the internal cambium in all the samples studied (Figs. 2A, 4E).

Development of internal cambium: In both the species, thin walled parenchymatous cells located between the protoxylem and internal protophloem underwent radial and tangential expansion (Figs. 2B, C, 4D) followed by subsequent divisions, which consequently resulted in the development of 4-6 small segments of internal cambium (Figs. 2C, D, 4F). These segments of internal cambia, although differentiated throughout the pith margin but they were never joined to form a complete cylinder of cambium (Figs. 2E-F, 3A, 4E). In *I. hederifolia*, initially all these cambial arcs were functionally unidirectional but after 2-3 weeks some of them became functionally bidirectional and began to produce xylem centrifugally and phloem centripetally (Figs. 2F, 3E). From these cambial segments, two of them located opposite to each other, became functionally more active and produced secondary xylem and secondary phloem elements (Fig. 3A, D). The rest of the segments produced secondary phloem only. Moreover, amount of secondary phloem produced by these segments were also relatively less as compared to the segments producing both secondary xylem and phloem. On the contrary, in *I. aquatica*, cambial arcs divided only on one side

producing only internal secondary phloem while there was no development of secondary xylem in it.

In both species of *Ipomoea*, the internal cambium was non-storied and composed of vertically elongated fusiform initials and more or less isodiametric ray initials. Cambial rays were mostly uni- to biseriate but 3-4 cells wide rays were also observed occasionally in mature stems. Like external cambium, fusiform initials were arranged in radial files of 3-6 cells (Fig. 3B-E).

Structure and development of intraxylary vascular elements: In both the species studied, internal cambium originated inside the protoxylem at the times when the plants reached flowering stage. Initially, it divided uni-directionally and only phloem elements differentiated centripetally. Production of xylem derivatives from the internal cambium was initiated only when maturation of fruits and dispersal of seeds was observed. Similar to the external phloem, intraxylary phloem was composed of sieve tubes, companion cells, axial and ray parenchyma cells (Fig. 3A, E). Sieve tube elements were relatively longer in internal phloem than in the external ones. It was 346 to 362 μm in length and 25 to 31 μm in width in internal phloem; while in the external phloem it was 323 to 368 μm and 23 to 27 μm in length and width respectively in *Ipomoea hederifolia* (Table. 2). In *I. aquatica,* it was measured 240 to 270 μm in length and 25 to 31 μm in width for internal phloem, and 157 to 231 μm in length and 22 to 27 μm in width for external phloem respectively (Table. 2). Phloem rays were mostly uni- to biseriate but occasionally multiseriate rays were also observed only in those segments of internal cambium that produced secondary xylem (Fig. 3E).

In the mature plants of *I. hederifolia*, differentiation of secondary xylem and phloem from the internal cambium exerted a pressure on pith cells pushing them towards centre and ultimately they were completely crushed (Fig. 3D, E). At the end of the growing season, considerable amount of secondary xylem and phloem was accumulated in the mature stems of all the samples studied. Xylem formed by the internal cambium was composed of vessels (both wider and fibriform), tracheids, fibre tracheids, axial and ray parenchyma cells. Vessels were mostly solitary but radial/tangential multiples of 2-3 members were also observed frequently. The alternate bordered pits on the lateral walls were oval to elliptic or oblong measuring

about 6-10 μm in diameter. Length and width of the wider vessel members measured from 228 to 438 μm and 134 to 248 μm respectively. Fibriform vessels were like imperforate tracheary element except for the occurrence of a small sub-terminal perforation plate near each end of the cell. In transverse view, it was difficult to distinguish these fibriform vessels from fibre tracheids and fibres. They were measured about 473 to 523 μm and 27 to 43 μm in length and width, respectively. Like internal phloem, rays in the secondary internal xylem were uni- to biseriate and occasionally contained multiseriate rays. They measured from 151 to 623 μm in height and 18 to 42 μm in width. Some of the samples also showed formation of intraxylary cork from the obliterated pith cells. No differentiation of such secondary xylem was observed in *I. aquatica.*

3.1.2 *Leptadenia reticulata* (Retz.) W. & A. (Asclepiadaceae)

Common Names: *Dodi, Shingutti, Hirandodi and Kharkhoda.*

Anatomy of young stem: In young stems of *Leptadenia*, the epidermis consisted of a single compact layer of isodiametric, thin walled parenchyma cells. A thin layer of cuticle covered the epidermis, while a 2-3 layered hypodermis composed of isodiametric thin-walled parenchyma cells differentiated beneath it. The bulk of the cortex composed of thin-walled parenchyma in which several immature laticifers were distributed randomly. However, the inner layer of cortex formed a discontinuous cylinder of thick walled, lignified perivascular fibres. An endodermis and pericycle was indistinct in the stem. The pith consisted of small thin-walled, isodiametric parenchyma cells and laticifers distributed randomly in the outer zone of the pith. Later on, as secondary growth progressed further, laticifers were also developed throughout the inner zone of the pith. The developmental anatomy of *Leptadenia* may be separated into three different types on the basis of primary and secondary growth. Primary growth comprises the development of internal phloem, while secondary growth consists of formation of included phloem (considered in unusual secondary growth section) and internal cambium, with differentiation of phloem in the pith.

Development of primary internal phloem: Proximal to the apical meristem, differentiation of primary internal and external phloem ensues in the first visible internode, which differentiated from semi-mature pith cells. Its differentiation started

simultaneously and preceded that of the protoxylem. Prior to the complete development and lignification of protoxylem derivatives, well-developed discrete strands of external and internal phloem occurred in the second visible internode (Fig. 5A, B). Gradually the bands of procambium became distinct, frequently interrupted by alternate bands of parenchyma cells (Fig. 5B, C). Procambial initials underwent tangential divisions to form radial rows of procambial cells and recent derivatives of protoxylem (Fig. 5C). The formation of procambial initials and protoxylem elements separated the external and internal phloem (Fig. 5D). At this stage the stem was composed of numerous conjoint and collateral vascular bundles consisting of external protophloem, protoxylem and internal protophloem.

The protoxylem was composed of xylem parenchyma and few elongated vessel members. These primary xylem elements possessed annular and helical thickenings with simple perforation plate on the slightly oblique to transverse end walls. Similar to the procambial initials, cells in the interfasticular region of the stem subsequently underwent tangential divisions, and formed new derivatives (Fig. 5D). At this stage, a narrow zone of actively dividing procambial initials internal to the external protophloem formed a cambium like meristem (i.e. metacambium). Tangential divisions of this meristem subsequently produced metaphloem centrifugally and metaxylem centripetally (Fig. 5D). The metaphloem consisted of sieve tubes, companion cells and small phloem parenchyma cells. The sieve tube members were relatively narrow and possessed simple sieve plates on their end walls, which were slightly oblique to transverse in its orientation. The metaxylem elements were arranged in radial rows (Fig. 5D) that were separated by uni- to biseriate parenchyma cells. Newly formed metaxylem elements consisted of tracheids, fibre tracheids, parenchyma cells and vessels. The first formed metaxylem vessel members were narrow and possessed helical or reticulate secondary thickenings. Their end walls tapered suddenly, with a very small simple perforation plate on their transverse to slightly oblique end walls (Fig. 5E, F). The diameter of these perforation plates was almost half the diameter of the vessel member. Later-formed vessel members may be narrower, with a simple perforation, and possessed small, opposite or alternate pits on lateral walls. Similar to the external phloem, intraxylary phloem was also composed of sieve tubes, companion cells, axial and ray parenchyma cells. Sieve tube elements were relatively longer in internal phloem than the external ones. It was measured

about 210 to 250 μm in length and 20 to 26 μm in width; while in the external phloem it was 200 to 225 μm in length and 18 to 24 μm in width (Table. 2).

Formation of internal cambium: In mature stems, when secondary growth was in progress, older non-functional internal phloem (sieve tubes) strands were replaced by the addition of new elements from adjacent parenchyma cells (Fig. 6A, B). As the internal phloem became non-functional, the sieve tube elements began to collapse; thus the space formed by obliteration of sieve tube elements was filled by the enlargement of adjacent parenchyma cells. Over a period of time, these parenchyma cells divided and differentiated into new sieve tube elements (Fig. 6C). In some of the samples, small arcs/segments of internal cambium were formed on the outer side of the internal phloem strands (Fig. 6D). This internal cambium was originated from the fully matured pith parenchyma cells by repeated tangential divisions. Parenchyma cells giving rise to the internal cambium were located along the outer margin of internal phloem strands. Functionally, the internal cambium was unidirectional in nature and cuts off only phloem elements centripetally. Structurally this phloem was more or less similar to that of other internal phloem cells. Like previous species (*I. hederifolia*) no differentiation of any xylem derivatives from this cambium was observed (Fig. 6D).

3.1.3 *Cressa cretica* Linn. (Convolvulaceae)

Common Names: *Rudravanti, Kardi, Lona* and *Khariyu.*

Anatomy of young stem: In the young stem of *C. cretica*, single layered compactly arranged thin walled isodiametric cells covered with thick cuticle composed the epidermis. Epidermal cells also bare unicellular trichomes while 2-3 layered hypodermis composed of chlorenchyma cells differentiated beneath it (Fig. 7A). The cortex was composed of parenchyma cells that showed accumulation of oval to circular, compound starch grains. In the mature plants, stems also showed heavy accumulation of druses in the parenchyma cells located external to the phloem.

Five vascular bundles arranged in the form of ring were interconnected by small arcs of interfascicular cambium and formed a continuous ring of the vascular cambium. As the secondary growth progressed further, characteristically pith became triangular in shape due to the pressure exerted by more and more intraxylary

secondary phloem (Fig. 7B). With the advancement of secondary growth, pith showed deposition of oval to circular and compound starch grains which were relatively less as compared to cortex. At this stage deposition of druses were also noticed throughout the inner zone of the pith.

Development of internal/intraxylary phloem: In *C. cretica*, differentiation of primary external protophloem precedes that of internal protophloem. Isolated strands of internal protophloem differentiate only after the formation of procambial initials. The procambial segments were frequently interrupted by alternate bands of parenchyma cells. At this stage, stem was composed of five vascular bundles comprised of external protophloem, protoxylem and strands of few internal protophloem elements in the third internode. Subsequently, tangential divisions occurred in the parenchyma cells located between the interfascicular regions that resulted in the development of a cylinder of the cambium like meristem. Periclinal divisions in this meristem differentiated metaxylem centripetally and metaphloem centrifugally (Fig. 7A). At this stage, patches of internal phloem differentiated from the procambially derived cells that were identified between first formed protoxylem elements and internal protophloem. These cells divided further and formed a group of small cells as compared to that of adjacent pith cells (Fig. 7E). These small cells served as a site for the origin of the internal cambium in future. Prior to origin of the internal cambium, additional internal secondary phloem elements were formed by the de-differentiation and re-differentiation of parenchyma located between the internal to the primary internal phloem and primary xylem (Fig. 7C, E). A considerable amount of internal phloem can be seen prior to development of the internal cambium in all the samples studied (Fig. 7C, E).

Development of internal cambium: Parenchyma cells located between the protoxylem and internal protophloem underwent radial and tangential expansion (Fig. 7E) following successive divisions and formed 3-5 small disconnected arcs/segments of internal cambium (Fig. 7B). From these segments, three arcs were dominant and functionally more active producing more phloem elements while other two segments got suppressed. These segments of internal cambia were separated by the bands of parenchyma cells situated at the pith margin (Fig. 7E, F). All these arcs were functionally unidirectional, and produced only secondary phloem centripetally (Fig.

7E, F). Structurally the internal cambium was non-storied and composed of vertically elongated fusiform initials and uni-biseriate, more or less isodiametric ray initials.

Structure and development of secondary intraxylary phloem: The internal cambium originated inside the protoxylem at the times when the plants were about 2.5 to 3 months old. This cambium is functionally unidirectional and produced only secondary phloem elements centripetally while no secondary xylem was produced even in the maximum thick samples available to us. Similar to the external phloem, intraxylary phloem was composed of sieve tubes, companion cells, axial and ray parenchyma cells (Fig. 7F). Sieve tube elements were relatively longer in internal phloem than in the external ones. It was 200 to 268 μm in length and 12 to 16 μm in width; while in the external phloem it was 140 to 187 μm in length and 17 to 24 μm in width (Table. 2). Phloem rays were mostly uni- to biseriate but multiseriate rays were observed seldom. In the mature plants, differentiation of secondary phloem from the internal cambium exerted a pressure on pith cells pushing them towards centre and pith becomes small triangular in shape and ultimately the pith was completely crushed (Fig. 7B).

3.1.4 *Solanum pseudocapsicum* Linn. (Solanaceae)

Common Names: *Jerusalem cherry, Christmas cherry* and *Madeira cherry.*

Anatomy of young stem: In the young stems, the epidermis was made up of a single layered compactly arranged thin walled parenchyma cells. A thin layered cuticle covered the epidermis externally while 2-3 cell layered hypodermis composed of parenchyma cells differentiated beneath it. The cortical parenchyma cells showed heavy accumulation of oval to circular, compound starch grains. Innermost part of the cortex also showed isolated bands of stone cells in the vicinity of external phloem. Pith cells were characterised by the presence of oval to circular and compound starch grains and calcium oxalate needles. Later on as secondary growth advanced, some of the pith parenchyma cells differentiated into stone cells, throughout the inner zone of the pith.

Development of internal/intraxylary phloem: Differentiation of primary internal and external phloem was seen in the first visible internode, which differentiated from semi-mature pith cells located at the margin of pith. Quantitatively internal phloem

cells are more in comparison with external phloem cells (Fig. 8A-D). Differentiation of internal protophloem started simultaneously with the external protophloem but it precedes that of the protoxylem. Prior to complete development and lignifications of protoxylem derivatives, well developed discrete strands of internal and external phloem occur in the third visible internode (Fig. 8C). Gradually the bands of procambium became distinct and frequently interrupted by alternate bands of parenchyma cells (Fig. 8A, B). With the advancement of primary growth, procambial initials underwent periclinal divisions and formed radial rows of procambial cells and recent derivatives of protoxylem (Fig. 8E). In the third internode, the young stem was composed of numerous collateral vascular bundles comprising of external protophloem, protoxylem and strands of few internal protophloem elements. Protoxylem was composed of xylem parenchyma and few elongated vessel elements. These protoxylem elements were characterized by annular and helical thickening with simple perforation plates on slightly oblique to transverse end walls.

In the sixth inter node, tangential divisions in the parenchyma cells located between the adjacent vascular bundles underwent de-differentiation and gave rise to interfascicular cambium. Segment of fascicular and interfascicular cambium were joined and formed a cylinder of the cambium like meristem called as metacambium. Tangential divisions in this metacambium consequently resulted into differentiation of metaxylem centripetally and metaphloem centrifugally. Metaxylem was composed of tracheids, fibre-tracheids, parenchyma cells and vessels. First formed metaxylem vessel members were relatively narrow and possessed helical or reticulate thickening. At this stage, patches of internal phloem became distinct and cells located between protoxylem and internal protophloem divided further and formed group of small cells as compared to adjacent pith cells (Fig. 8D, E). Prior to the origin of the internal cambium, these cells underwent repeated divisions and differentiated into additional internal secondary phloem. Similar to external phloem, internal secondary phloem was composed of sieve tubes, companion cells and parenchyma. Considerable amount of internal phloem was accumulated prior to development of the internal cambium in all the samples studied (Figs. 8D, E, 9B).

Structure and development of internal cambium: As the secondary growth proceed further, older non-functional sieve tube elements from the internal phloem were

replaced by the addition of new sieve elements from the parenchyma cells situated between protoxylem and internal protophloem (Fig. 8D, E). When internal phloem became non-functional, the phloem strands showed heavy accumulation of callose on the sieve plates followed by the collapse of sieve tube elements (Fig. 9A, E). Therefore, the space formed by obliteration of sieve tube elements was occupied by the enlarging the adjacent parenchyma cells. These enlarged parenchyma cells divided repeatedly and formed additional sieve tube elements (Fig. 9C-F) and small segments of internal cambium (Fig. 9E, F). These segments of internal cambium were situated along the outer margin of internal phloem strands. Functionally these arcs of internal cambium were unidirectional, and produce only secondary internal phloem centripetally. Structurally this phloem was similar to the external phloem produced by cambium (Fig. 9E, F). It was composed of sieve tubes, companion cells, axial and ray parenchyma cells. Phloem rays were mostly uni- to biseriate. Sieve tube elements were relatively longer in internal phloem than in the external ones. It was 229 to 280μm in length and 23 to 28 μm in width; while in the external phloem it was 220 to 269 μm in length and 19 to 27 μm in width (Table. 2).

3.1.5 *Strychnos bicolor* Prog. (Loganiaceae)

Structure and development of the young stem: In the young stems, 4-6 layered cork cells replaced the epidermis and formed outermost layer but as the secondary growth progress further, number of cells in the periderm region increased (2-3 mm in thickness) in the 20 mm thick stems (Fig. 10A, B). These cells were characterized by relatively wide lumen with thick, sclerosed walls on peripheral side of the stem. Oval to polygonal parenchyma cells beneath periderm composed the cortex with a continuous ring of sclereids and can be found in the middle of it. In the thicker stems, the cortical parenchyma became tangentially elongated due to increase in the stem diameter that exerted a pressure on the cortical parenchyma (Fig. 10B). Therefore, a continuous ring of stone cells was also fragmented but its continuity was retained by the re-differentiation of adjacent cortical cells into new stone cells. Pericycle consisted of rod shaped elongated cells which were not much distinct but can be identified by the isolated strands of pericyclic fibres. The central portion of the stem was composed of thin walled parenchymatous pith. In the centre, pith cells were often intermingled with group of more or less isodiametric stone cells in the centre of pith.

Peripheral portion of the pith was also characterised by the presence of isolated bundles of intraxylary phloem. Abundance of prismatic/rod shaped crystals were observed in the parenchyma cells of the pith as well as in the cortical region of all the samples studied.

Development of intraxylary phloem: Young stems of *Strychnos* showed presence of intraxylary phloem from the beginning of primary growth. In mature stems, older nonfunctional sieve tube elements of internal phloem strands were replaced by the addition of new elements from adjacent pith cells (Fig. 11D, E). As the sieve tubes of internal phloem became nonfunctional, the strands begin to collapse; thus the space formed by obliteration of sieve tube elements is filled by the enlargement of adjacent parenchyma cells. Over time, these parenchyma cells divide and differentiate into new sieve tube elements (Fig. 11E). In some samples, small arcs/segments of internal cambium were formed on the outer side of the internal phloem strands (Fig. 11E). This internal cambium was originated by the de-differentiation of fully matured pith parenchyma cells. Parenchyma cells that gave rise to the internal cambium were located along the outer periphery of internal phloem strands. The internal cambium divided uni-directionally and cuts off only phloem elements centripetally. Structurally this phloem was more or less similar to that of interxylary and external phloem and was composed of sieve tubes, companion cells, axial and ray parenchyma cells (Fig. 11D, E). Like normal cambium, the internal cambium was structurally composed of vertically arranged fusiform cambial cells and radially elongated uni- to multi-seriate rays (Fig. 11E). Sieve tube elements were relatively longer in internal phloem than in the external ones. It was 375 to 389 μm in length and 26 to 30 μm in width; while in the external phloem it was 364 to 374 μm in length and 22 to 28 μm in width respectively (Table. 2).

3.2 MEDULLARY BUNDLES

In the stems of the some plants, supplementary vascular bundles occur in the pith and these are termed as medullary bundles (Philipson and Ward 1965; Metcalfe 1983). Wilson (1924) investigated the medullary bundles which are common in families like the Amaranthaceae and the Chenopodiaceae. However, according to Eames and McDaniels (1947), these bundles are observed less frequently in ferns, as in *Pteridium*. In certain dicotyledons families, medullary bundles are present in addition

to the normal cylinder of bundles, such as the Cactaceae and the Nyctaginaceae and these medullary bundles can be considered as anomalous structure type (Eames and McDaniels 1953; Cutter 1969; Lopes *et al.* 2008). Perusal of literature indicates that in dicotyledons, medullary bundles occur in a considerable number of families such as the Piperaceae, the Ranunculaceae, the Amaranthaceae, the Berberidaceae, the Cucurbitaceae and the Convolvulaceae (Gangulee *et al.* 1988; Pant and Bhatnagar 1975). In the Nyctaginaceae and the Piperaceae, many bundles occur in the pith which are either arranged in the form of rings or may be distributed irregularly (Gangulee *et al.* 1988). Medullary bundles are closely spaced when initiated near the shoot tip, but as the shoots continue growing, the pith expands and medullary bundles are pushed to a wider spacing, with very low densities in the older trunks (Mauseth 1993). Medullary bundles are observed commonly in the members of the Cactaceae and the Nyctaginaceae. However, the role of these bundles is completely unknown (Mauseth 1988a). According to Park (2002), in Cactus medullary bundles play important role in translocation of water and starch by forming broad pith, because they continue to produce phloem throughout the life of the plant and starch is abundant in many pith sections. In the present study, structure and development of medullary bundles is studied in the stems of *Argyreia nervosa* (Burm. f.) Bojer, *Mirabilis jalapa* L. and *Boerhaavia diffusa* L. is described in detail.

3.2.1 *Argyreia nervosa* (Burm. f.) Bojer (Convolvulaceae)

Common Names: *Elephant creeper, woolly morning glory* (U.S.A.), *Samundarsok* (Gujrati) and *Sagarvell* (Marathi).

Anatomy of young stem: In the young stem of *Argyreia*, a thin layer of cuticle covers the epidermis. The epidermis was made up of single compact layer of thin walled isodiametric parenchyma cells. Two to three layered hypodermis composed of thin walled parenchyma cells differentiate beneath it. The mass of cortex was composed of thin walled oval to polygonal parenchyma in which several immature laticifers were distributed randomly (Fig. 12A). However, the inner layer of cortex does form a discontinuous cylinder of thick walled, lignified perivascular fibres. The pith consists of thin walled, small isodiametric parenchyma cells. At this stage, laticifers were distributed randomly in the outer zone of the pith (Fig. 12A). Later on,

development of laticifers was observed throughout the inner zone of the pith (Fig. 12B).

Development of procambium: Several collateral vascular bundles became apparent from the apical meristem about 500 to 800 μm away from the apex. The cell that differentiated into procambium were characterised by dense cytoplasm and distinct large nucleus. These cells also took dark staining and were devoid of vacuoles. In transverse view, the promeristem differentiated into different parts of young shoot while few cells retained their meristematic ability and differentiated in to procambium. This procambium was functionally bidirectional and gave rise to protoxylem centripetally and protophloem centrifugally (Fig. 12A). After formation of few primary vascular elements, procambium was transformed into metacambium and gave rise to metaphloem centrifugally and metaxylem centripetally.

Development of medullary bundles: *Argyreia nervosa* is characterised by the presence of medullary bundles along with few celled strands of internal phloem (Fig. 13A, B). Development of medullary bundles took place more or less simultaneous with the development of protoxylem and protophloem. In transverse sections, some of the thin walled immature pith cells with dense cytoplasm with dark staining acquired meristematic activity. These meristematic cells differentiated into protoxylem and protophloem on either side of it. In the young stem (i.e. 3 node), the pith had a diameter of 6.0 mm and possessed 20 medullary bundles; the increases, slightly older pith (i.e. in the 5^{th} node) had a diameter of 16 mm and had 26 medullary bundles. Apparently medullary bundles were initiated only very near the apical meristem.

In the beginning, when medullary bundles began to differentiate they were closely spaced i.e. with a range of 5 to 9 bundles/mm^2. However, as the shoots continued to grow, diameter of the pith expanded and medullary bundles were pushed apart from each other to a wider spacing, ranging from bundles 25 to 30 per mm^2 in old stems (i.e. in the 20^{th} node). As the secondary growth progresses further in relatively old plants, the pith had about 25 to 35 bundles per mm^2 bundle. These bundles were arranged in the perimedullary ring in the young stem but or they appear irregularly scattered in the thick stems due to additional development of medullary bundles throughout the pith.

In young shoots, the medullary bundles were developed opposite to the protoxylem elements of the normal/regular vascular bundles. In the young shoot, medullary bundles were arranged in the form of ring and most of them were arranged inversely i.e. protoxylem facing outwards and phloem towards the centre of the pith. The earliest stage in their ontogeny is indicated by meristematic activity in the parenchymatous cells near the periphery of the pith. These cells showed characteristic of meristematic cells i.e. prominent nuclei and densely stained cytoplasm. Anticlinal, periclinal, and transverse divisions took place rapidly, and formed group of cells in the second internode, which differentiated into laticifers. In the 3rd internode, some of the parenchyma cells adjacent to differentiating laticifers began to differentiate into phloem elements. Development of the phloem in the medullary bundles parallels that of the phloem in the vascular bundles of the petiole. However, differentiation of laticifers precedes that of medullary bundles and distinctly differentiated medullary bundles may be seen in the 4th inter node. At this stage, most of the bundles possessed only phloem elements while protoxylem elements with only one or two protoxylem derivatives was observed occasionally.

As the stem matures, the active parenchymatous cells surrounding the phloem cylinder differentiated as a ring of cambium cells with two to four cells arranged in radial files. This newly formed cambium underwent cell division activity and formed secondary xylem and additional phloem elements are differentiated. The secondary xylem consists primarily of fibers, but in most bundles vessels and tracheids were also observed. These vessel or tracheids were distributed irregularly or scattered among the fibers. The xylem derivatives produced from these cambia were arranged in radial rows. The fibers were characterized by tapering ends and thick walls, with very small but abundant simple pits. Secondary wall thickening of the xylem elements is primarily of the scalariform type but some of the vessel elements also showed reticulate thickenings.

In the mature stems, the medullary bundles were relatively larger in size due to addition of more xylem derivatives by the activity of medullary cambium. With the advancement of secondary growth in the stems, many bundles were observed to arise de novo and terminate in the pith. Pattern of medullary bundles development was not fixed and it was differed from sample to sample and sometime variations were observed within the same section. For example, delayed development of xylem results

in only presence of phloem bands while xylem remains absent, in later stage a xylem parenchyma cells on the periphery of these phloem bands became meristematic in nature and produce secondary xylem from all sides thus resulting into amphivasal bundles. In some of the sections, these bundles did not form complete amphivasal arrangement and it becomes semiconcentric or forming "C" shaped arc of thick walled xylem elements encircling the phloem patch. Sometimes, the cambium may develop only on one side of the phloem band and it became collateral vascular bundle. In another situation, pith cells underwent repeated divisions and became meristematic in nature. From which, central cells differentiated into lignified elements and marginal cells differentiated into phloem elements the thus the resulting vascular element became bicollateral. Occasionally, some of the vascular bundles showed biamphicrible arrangement i.e. a phloem band is surrounded by thick walled lignified fibres and tracheids (arrangement like amphicribal vascular bundle) and this amphicribal vascular bundle is again encircled by a phloem band from all sides.

The bundles do not have any specific orientation pattern, as mentioned earlier; most of the medullary bundles located at the periphery of the pith were inversely oriented while rest of them did not have any specific orientation. Moreover, their number varies from different parts of the stem due to fusion or separation. Furthermore, same bundle may show all different types of arrangement mentioned in above paragraph at different levels of the stem.

3.2.2 *Mirabilis jalapa* L. (Nyctaginaceae)

Common Names: *Four O'clock, Beauty-of-the-night* and *Gulbakshi.*

Anatomy of young stem: In young stems, several collateral vascular bundles become connected by interfascicular cambium and formed a continuous ring of cambium (Fig. 14A). After the development of 25-30 xylem derivatives, only small segments of cambium ceased to divide while alternating segments remain functional. A new segment of cambium was developed from the parenchyma cells outside the phloem to replace these nonfunctional segments, and formed a complete ring (Fig. 14B). Cessation of cell division is observed only in segments of vascular cambium that were producing conducting elements of secondary xylem and phloem; continuous activity is observed in the alternating segments that produced only conjunctive tissues on both

the xylem and phloem sides. The segments of cambium that ceased to divide usually maintained a radial arrangement of cambial cells, but occasionally the entire segment differentiated into its derivatives (Fig. 14A, B). In such cambial segments, the phloem elements were directly in contact with the xylem derivatives, as in monocots (Fig. 14B). The vascular cambium was semi-storied and composed exclusively of relatively short fusiform cambial cells (Fig. 14 D). Fusiform cambial cells were 220-260 µm in length and 22-24 µm width (Table. 3a). In actively dividing cambium, the cambial zone is 3-5 cells wide; non-dividing cambial segments embedded in the xylem due to cessation of their activity are 2-3-cell layered in each radial file.

Origin and development of medullary bundles: In *Mirabilis jalapa* stem development of medullary bundles occurred simultaneous with protoxylem elements and remained functional till completion of life cycle of the plant. Many small medullary bundles originated near the apical meristem. In transverse sections, some of the thin walled cells located in the pith region having dense cytoplasm with dark staining acquired meristematic activity and began to differentiate into protoxylem and protophloem, which ultimately differentiated into medullary bundles. Initially they showed less protoxylem and more protophloem, in some plants they lack tracheary elements initially. As the secondary growth progressed further, these medullary bundles were more distinct due to deposition of additional secondary xylem and phloem elements (Fig. 14A-C). In the thick stems, the medullary bundles were highly variable in size; they were as large as normal vascular bundles in the outer ring while some of them were only a few celled (Fig. 14C). The smaller bundles may consist entirely of phloem, while others have extensive phloem development with several tracheary cells. The number of bundle is highly variable due to merging and splitting of these bundles at various levels of the stem. In the young stems these medullary bundles were closely spaced near the shoot tip i.e. with a range of 6 to 10 bundles/mm^2, but with advancement of extension and radial growth, the pith enlarges due to complete differentiation of pith cells. Thus, the medullary bundles were pushed apart from each other, in mature stems (i.e. in 4-6 mm thick), they were ranging from 25 to 30 per mm^2, with very low densities in the older stem (i.e. in 15-20 mm thick).

The vascular cambium present between the xylem and phloem of the medullary bundles maintained its radial alignments even at the senescent stage of the

stem and remained functionally active. However, the rate of cell division and differentiation of xylem and phloem was rather very slow thus, did not show any significant increase in the size even at the senescent stage of the stem. The cambium of medullary bundles was 3-4 layered in transverse view and showed bi-directional differentiation of xylem and phloem. In an entire medullary bundle, vessel wall thickening showed wide range of variation from helical to alternate bordered pits with transitional forms between them representing primary to secondary xylem. As compared with the outer secondary xylem, the length and width of vessel members was relative less measuring from 190 to 215 μm and 35-65 μm respectively (Table. 3a). Phloem close to the cambial segment remained functional even at the senescent stage of the stem. Structurally the phloem of the medullary bundles remained same with that of phloem produced by vascular cambium. The non-functional sieve tube elements away from the cambial segment were characterised by heavy accumulation of callose followed by obliteration of the sieve tune elements; while functional sieve tube elements possessed open sieve pores even at the senescent stage.

3.2.3 *Boerhaavia diffusa* L. (Nyctanthaceae)

Common Names: *Hog Weed, Pig Weed, Horse Purslane, Tar Vine* and *Punarnava.*

Anatomy of young stem: The young stem of *Boerhaavia diffusa* was covered with glandular trichomes while epidermis was composed of single compact layer of isodiametric, thin walled parenchyma cells covered with thin layer of cuticle. The cortex consisted of 2-3 cell layered collenchyma cells followed by 2-3 layers of thin walled parenchyma cells while an endodermis was indistinct and pericycle is very narrow, consisting of only one or two cell layers. Initially in the pith, there were only two large central bundles, then a loose ring of bundles outside these, and finally an outer ring of bundles much smaller than the rest, but far more in number and situated just beneath the pericycle. The pith was composed of thin walled, isodiametric parenchyma cells and medullary bundles were distributed in the pith.

Structure and development of medullary bundles: Medullary bundles were initiated close to the apical meristem. Initially, there were two large central pith bundles embedded in parenchyma, then a loose ring of bundles outside these. After sometime, these two bundles becomes a medullary bundle and loosely arranged ring

of bundles forms developing procambial strands results into production of protoxylem centripetally and protophloem centrifugally. In transverse sections stele bundles had only protoxylem, medullary bundles were also present and also had protoxylem. These two central medullary bundles were the largest bundles in the stem, and had a tangential diameter about twice as long as the radial. As growth progressed new medullary bundles were originated and added into pith. These medullary bundles were closely spaced when initiated, with a range of 4 to 6 bundles in small pith (Fig. 14E). As the shoots continued to develop, the pith expanded and medullary bundles were pushed to a wider spacing, but very few additions of medullary bundles. In matured stem, new medullary bundle developed from peripheral pith cells (Fig. 14F). After some time medullary bundles were expanded and united in such way that they covered entire pith. At that time, there was no further origin of new medullary bundles.

In the mature stems, the vascular cambium present between the xylem and phloem of the medullary bundles maintained its radial alignment even at the senescent stage. As secondary growth progressed, the earlier phloem cells become crushed leaving a cap of dead phloem cells over the later formed phloem lying beneath. Cell division and differentiation of xylem and phloem from this cambium occurred very slowly, with no significant increase in the size of the medullary bundle even in the 15-18 mm thick stems. Dimensionally, the xylem and phloem elements of medullary bundles were more or less similar length and width to that of other vascular elements produced by external cambium (Fig. 14E, F).

3.3 CORTICAL BUNDLES

Presence of vascular bundles in cortex termed as cortical bundles. Holroyd (1928) consider such bundles as accessory vascular bundles found in cortex of certain plants belonging to the families Melastomaceae, the Calycanthaceae and in the Cactaceae. There is no unanimous opinion regarding the nature and function of the cortical bundles whether they are leaf trace bundles or vascular bundles other than leaf traces. According to Smith (1928) cortical bundles are leaf trace bundles which pursue an independent course through the cortex without entering the ring. This opinion is also supported by Fahn and Bailey (1957), Tiag (1963) and Metcalfe and Chalk (1983). Anderson (1962), in his work on *Petradoria*, used the term "cortical bundles" to refer

to leaf traces passing through the cortex. Usage of this term is contrary to that of other authors. According to Karrfalt (1975) cortical bundles are vascular bundles other than leaf traces found in the cortex of the stems in members of numerous dicotyledonous families. Beck (2010) stated that an unusual distribution of primary vascular bundles, especially the occurrence of cortical bundles in dicotyledons is one of the anomalous characters. According to him, these bundles are scattered vascular bundles exterior to main vascular cylinder (*Cucurbita* and *Piper betle*) as well as leaf traces in some taxa that extend over great longitudinal distances in the stem prior to entering the leaves for example *Piper excelsum.*

Metcalfe and Chalk (1950) listed about 37 families showing presence of cortical bundles. In few of them cortical bundles have been studied thoroughly, and some of the reports are apparently erroneous (Fahn 1967; Karrfalt 1975). It is considered that cortical bundles may be common to systematic units of various ranks. In *Calycanthus* and *Chimonanthus* of the Calycanthaceae have similar cortical-bundles, as do certain genera of the Chenopodiaceae (Fahn and Bailey 1957; Fahn and Arzee 1959). In some cases, cortical bundles may be present in only a single species of a family, such as *Nyctanthes arbortristis* member of Oleaceae (Majumdar 1941). Cortical bundles are diagnostically significant because of their taxonomically restricted occurrence while there is no clear evidence that the cortical bundles of the Compositae are characteristic of taxonomic categories higher than the genus (Karrfalt 1975).

Metcalf and Chalk (1950) report about 24 genera of the Compositae with cortical bundles. Fifteen of these are included as a result of their original observations, and nine follow Solereders earlier work (1908). Most of the older references to cortical bundles cited by Solereder are extremely brief and only incidental to the works in which these appear. One report (Heinricher 1883) is explicitly devoted to cortical bundles in *Centaurea* and goes into some detail correlating the development of the cortical bundles with that of the assimilatory tissues in the stem. Some dicotyledons show scattered vascular bundles similarly some monocots show their arrangement in the form of ring. For example, *Thalictrum* of the family Ranunculaceae, in *Podophyllum* of the family Berberidaceae and *Papaver* of the family Papaveraceae show scattered vascular bundles which belongs to dicot. On the other hand, *Tamus communis* of family Dioscoreaceae is a glaring example of a

monocotyledon with bundles arranged in ring like fashion toward periphery round large central pith (Eames 1961; Singh 2004). Particularly inverted bundles are also noticed in plants like *Rumex crispus* and *Rheum officinale* of family Polygonaceae where phloem instead of xylem occurs toward the pith in the primary bundles (Woodcock 1914; Joshi 1936; Carlquist 2003). In plants with fleshy cortex, such as many members of the Cactaceae, where the leaves are reduced and photosynthesis is carried out largely by the cortex, show presence of cortical bundles.

According Mauseth and Sajeva (1992) and Park (2002) cortical bundles appear to be involved in three processes: i) transporting photosynthate from the outer, chlorophyllous palisade cortex to the stele; ii) transporting sugars to and from storage cells in the inner, nonphotosynthetic cortex; and iii) transporting water throughout the cortex. Phloem in cortical bundles is probably involved in sugar transfer when the cortex acts as a starch storage tissue. Now therefore, question arises that whether these bundles are accessory bundles? Are they playing any important role in translocation of food materials in other dicotyledons (except in Cactoideae)?

Therefore, in present study development of cortical bundles is studied in *Couroupita guianensis* Aubl., and *Nyctanthes arbortristis* L. Occurrence of cortical bundles in *Couroupita guianensis* Aubl., is reported for the first time in the present investigation while *Nyctanthes arbortristis* L., is reinvestigated to verify their functions.

3.3.1 *Couroupita gyuianensis* Aubl. (Lecythidaceae)

Common Names: *Naga linga*, *Cannon Ball tree* and *Brazilian Nut.*

Anatomy of young branches and peduncles: In the young branches (3rd visible node), epidermis was composed of barrel shaped epidermal cells which enclosed the 2-3 layered hypodermis. A cortex was composed of 8-12 layered thin walled parenchyma cells in which several irregularly oriented cortical bundles were distributed randomly. In relatively thick stems, size of these cortical bundles differed depending on their age and distance of origin. After completion of primary growth, several collateral vascular bundles were joined by interfasicular cambium and formed a complete cylinder of the vascular cambium. Functionally this cambium was

bidirectional producing secondary xylem centripetally and secondary phloem centrifugally.

Development of cortical bundles: Young branches of *Couroupita* were charactarised by the presence of cortical bundles of irregular size and orientation (Figs. 15A, B, 16D). Development of cortical bundles was initiated close to the apical meristem. As the diameters of the young stems were narrow, initially they were closely spaced with a range of 5 to 9 bundles per mm^2. However, as the shoots continued to develop, the cortex expanded and cortical bundles were pushed to a wider spacing (Fig. 16A-D), with a range of 31 to 36 bundles per mm^2 in older stems. In the very young apices it is difficult to detect these bundles as they are the part of the normal vasculature which gradually gets separated from the ring of vascular prior to formation complete ring of vascular cambium. During the primary growth, promeristem began to differentiate protophloem and protoxylem elements in the 2^{nd} and 3^{rd} visible internode. Thereafter, strands of procambium appeared in between protoxylem and protophloem and resulted into formation of several conjoint and collateral vascular bundles. These vascular bundles were arranged in the form of ring. With the advancement of extension growth, in the 5^{th} visible internode all these vascular bundles were interconnected by forming interfascicular cambium (i.e. metacambium stage). At this stage, two vascular bundles about 45 ° angles were not in the same line/plane with others and they were relatively pushed outside (Fig. 16A-C). Thus the ring of cambium was interrupted at these points. However, separation and pushing of the vascular bundle starts prior to formation of complete ring of the cambium, thus it becomes difficult to detect the separation of these bundles. With the advancement of secondary growth, these bundles also underwent secondary thickening by deposition of secondary xylem and phloem elements.

Pattern of medullary bundles development was not fixed and it was differed from sample to sample and sometime variations were observed within the same section (Fig. 15C-F). For example, they may be conjoint and collateral or sometimes only phloem was formed while xylem remained absent (Fig. 16E). Occasionally, the cambium may develop on both side of the xylem thus it became bicollateral vascular bundle (Fig. 15D). Structurally, secondary xylem of the cortical bundles was composed of vessel elements, fibres, tracheids, and axial and ray parenchyma cells.

Length of the tracheary elements was similar to the normal secondary xylem produced by the cambium while its diameter was relatively less. The phloem was composed of sieve tube members, companion cells, axial and ray parenchyma cells.

3.3.2 *Nyctanthes arbortristis* L. (Nyctanthaceae)

Common Names: *Night-flowering Jasmine*, *Coral Jasmine*, *Paarijat*, *Prajakt* and *Nala kumkumaka.*

Anatomy of young branches: Young stem of *Nyctanthes* was quadrangular in shape, covered with unicellular trichomes and glandular trichomes (Fig. 17A, B). Epidermis was made up of single layer of more or less isodiametric, thin walled parenchyma cells and was covered with thick layered cuticle. A 2-3 layered hypodermis composed of chlorenchyma cells differentiated beneath the epidermis (Fig. 17A, B). The hypodermis was followed by parenchymatous cortex. Innermost part of cortex in the young stem showed continuous band of pericyclic fibres while the pericyclic fibre band was interrupted in the mature and thick stem due to enlargement of stele due to deposition of secondary xylem and phloem. Endodermis and pericycle was indistinct. Four cortical bundles were distributed in the cortex region at corners of the quadrangular stem (Fig. 17A). These bundles were conjoint, collateral and placed inversely i.e. phloem occurred toward the pith instead of facing towards the cortex. A "C" shaped sclerenchymatous cap encircled the phloem of the cortical bundle (Fig. 17B, F). On the other hand vascular bundles arranged in the form of ring were interconnected by interfascicular cambium and formed a continuous ring of the vascular cambium.

Development of cortical bundles: Four cortical bundles located at four corners of quadrangular stem initiated close to apical meristem (Fig. 17A). Being associated with leaves, these bundles originated simultaneously with the differentiation of leaf primordium. At corners of quadrangular stem, when the procambium was in the process of development, some of the cells outside to it underwent repeated divisions and formed sieve tube elements followed by protoxylem elements. Differentiation of protoxyleme elements in the cortical bundles began when a complete ring of vascular cambium was formed in the main stem. In these bundles, differentiation of phloem preceded that of the phloem. As compared with normal vascular bundles, cortical

bundles were inversely oriented; size of the cortical bundles increases with the increase in girth of the stem but number of bundles remained same even in the thick stem. The phloem part of these bundles was composed of sieve tube members, companion cells, and axial parenchyma while the xylem was composed of vessel elements, fibres, parenchyma and tracheary elements. Tracheary elements in cortical bundles were more or less similar to the tracheary elements produced by the normal vascular cambium while they were narrower than corresponding tracheary elements formed by the cambium in the same transverse section (Fig. 17B-D).

II. Unusual Secondary Growth

3.4 SUCCESSIVE CAMBIA

Successive cambia are familiar as the cause of concentric rings in Beet. They occur as continuous/complete rings or in the form of bands or strands of secondary phloem and secondary xylem embedded in a parenchymatous or fibrous (conjunctive tissue) background (Carlquist 1988, 2007a, b). According to Carlquist (2001a), successive cambia are known to occur in 34 families of dicotyledons (number of families varies with taxonomic practice). Metcalf and Chalk (1983) termed this phenomenon as "concentrically alternating xylem and phloem" and suggested that the term "interxylary phloem" or "included phloem" is applicable where successive cambia are involved. Carlquist (1988) suggested that use of term interxylary phloem to a type where a single cambium is involved. Schenck (1893) and Pfeiffer (1926) used the term successive cambia; the latter one is more accurately applicable to many of the genera in which the phenomenon is known today. Included phloem are terms applied to the phloem produced centripetally from the cambium or externally from isolated cambial segments within the secondary xylem as in *Strychnos* and in several Bignoniaceae species (Mikesell and Popham 1976; Carlquist 1988; Rajput *et al.* 2009). The term "included" is a misnomer in the case of dicotyledons with successive cambia because conjunctive tissue in those species is either formed as a background tissue or as bands between one vascular band and another. By definition the phloem is thus not "included" within secondary xylem in species with successive cambia (Carlquist 2002). According to him, the term included phloem is not to be confused with the phloem produced by successive cambia. In the later, phloem is produced outwardly from each of the successive cambia and therefore lies between conjunctive tissue (which is not secondary xylem) and the secondary xylem produced by each cambia. Thus in dicotyledons with successive cambia, the phloem are neither "interxylary" nor "included". Interxylary phloem, on the contrary, is produced centripetally or centrifugally by a single cambium and thus occurs as strands intercalated in a secondary xylem background. Stevenson and Popham (1973) pointed out that the secondary phloem of successive cambia is not included within wood at all.

Maintenance of such terms may have been furthered by those who are involved with wood identification (IAWA Committee 1989) and who therefore want simple terms. The distinctive appearance of successive cambia and their products can easily be learned by wood anatomists. However, they desire to use such a term as "included phloem" probably indicates a desire to consider the background tissue of plants with successive cambia as "wood", but although often woody in texture (Carlquist 2007a). This background tissue is not wood in the ordinary sense. Wood anatomists who have dealt in detail with plants with successive cambia have used the term conjunctive tissue for the background of fibres and/or parenchyma in which vascular increments are embedded (Carlquist 2007a). Terminology is only one symptom of the problems involved in analysis of successive cambia and their products. Although various authors describe the histology of species involved with reasonable accuracy, understanding of the ontogeny of successive cambia has been troublesome (Carlquist 2007a). One of cause again described by Carlquist (2007a) is that soft and hard tissues are intermixed in stems and roots with successive cambia. Soft tissues and stages in their development are frequently damaged and uninterruptable when such untreated axes are sectioned on a sliding microtome (Carlquist 1982).

The successive rings are characterized by formation of xylem centripetally and phloem centrifugally. However, in some plants, every alternating cambium ring produces secondary phloem centripetally and secondary xylem centrifugally. In the present investigation, such cambia are referred as inversely oriented cambia. This pattern of secondary growth is reported for the first time in *Ipomoea hederifolia*, *Ipomoea biloba*, and *Ipomoea quamoclit*, whereas *Dolichos lablab*, shows isolated bundles having inverse orientation. Formation of successive cambia has been known since long and tends to occur in different members of the Convolvulaceae (Metcalfe and Chalk, 1950; Pant and Bhatnagar, 1975; Lowell and Lucansky, 1986; Carlquist and Hanson, 1991). Various workers have studied formation of successive cambia in the Convolvulaceae; among them the most important contributions are those of Metcalfe and Chalk (1950), Mennega (1969), Lowell and Lucansky (1986), Carlquist and Hanson (1991). Successive cambia also occur in stems and roots of some familiar plants such as *Amaranthus, Atriplex, Bougainvillea, Chenopodium, Cycas, Mirabilis, Phytolacca, Welwitschia* and so on. These successive cambia occur in the flattened or

variously shaped forms of the giant lianoid stems of *Bauhinia, Gnetum,* and numerous members of the Menispermaceae. Although, Caryophyllalean families (especially the "Centrospermoid" families) form a prominent nexus on the list, successive cambia must have arisen in about 15 other clades. *Welwitschia* and all species of *Gnetum* (Carlquist 1996a) contain successive cambia, and they have been reported in several species in each of the cycad genera such as *Cycas, Encephalartos,* and *Macrozamia* (Greguss 1968). In the present study, formations of successive cambia have been studied in families such as the Chenopodiaceae (recently merged with Amaranthaceae by Angiosperm Phylogeny Group), the Convolvulaceae, and the Menispermaceae. For the sake of convenience development of successive cambia is further bifurcated into: i) successive cambia having normal orientation and ii) successive cambia having inverse orientation.

3.4.1 Successive Cambia Having Normal Orientation:

3.4.1.1 *Spinacia oleracea* Linn. (Chenopodiaceae)

Common Names: *Spinach* and *Palak.*

Structure of secondary xylem: Both, stems and roots of the mature plants were composed of successive rings of xylem alternating with phloem (Fig. 18A). Xylem was mainly composed of vessels, tracheids, libriform fibres and thin walled parenchymatous conjunctive tissue. Xylem fibres were 378-413 μm in length and 18-22 μm in width. In the secondary xylem, thin walled parenchyma cells were relatively more abundant as compared to that of thick walled lignified elements such as vessels, tracheids and fibres. These parenchymatous conjunctive tissues showed deposition of secondary wall material and became thick walled. However, even after depositing secondary wall material (lignin), these cells retained their nucleus. Morphologically these conjunctive parenchyma cells appeared like nucleated xylem fibres but they possessed simple circular pits on their wall. Moreover, they differed from fibres in being wider in diameter and shorter in length. Most of them had abruptly tapering ends while others appeared like axial parenchyma cells (Fig. 18B). Length of these cells measured from 256-288 μm and was about 1.5 to 2.5 times longer than the fusiform cambial cells (159 to 210μm). Length and diameter of the vessel elements was 98-178 μm and 61-73 μm respectively. Not much variation was observed in the xylem structure of both stems and roots except the thick walled lignified tissues were

relatively more abundant in the stems than in the roots. In both roots and stems xylem was found devoid of typical ray cells (Fig. 18B). In longitudinal sections, some of the cells were small, spindle shaped and appeared like ray cells but when they were traced in radial view there was no continuation of these cells in radial direction. In radial view these ray like cells were more or less similar in dimension as they were seen in tangential view. Similarly, in transverse view, there were groups of parenchymatous (conjunctive tissue) cells arranged in radial rows appearing like ray cells. One can even trace these radial cell rows from one tissue ring to another (Figs. 19A, B). These cells separate the bundles of conducting elements of xylem and phloem from the conductive tissues (Fig. 19C). In tangential and radial view, these cells did not appear like ray cells but rather they were axially elongated parenchyma cells.

Structure and development of vascular cambium and its derivatives: The semi-storied cambium of *Spinacia* was exclusively composed of vertically elongated fusiform cambial initials with no rays even in the mature stems and roots (Fig. 18C). The cambia became 2-3 layered when resting and 3-5 layered during active period of growth (Fig. 18D). Length and width of the fusiform cambial cells were measured to be 159 to 210 μm and 17 to 21 μm respectively.

Secondary growth in the *Spinacia* was achieved by the simultaneous development of multiple rings of successive cambia (Fig. 18D). Each cambium ring was originated from the parenchyma cells located outside to the phloem produced by the previous cambial ring (Fig. 18D, E). The cambium divided bi-directionally and formed secondary phloem centrifugally, and conjunctive tissue and secondary xylem centripetally. Even though very few xylem derivatives were differentiated from the earlier cambium, a new ring of cambium was originated outside to the previous cambium (Figs. 18D, 19A, B). In each newly developed cambium, formation of phloem precedes that of xylem (Fig. 18E). Simultaneously 2-3 rings of cambia were found active at a time throughout the stems and roots in all the samples studied (Fig. 19C). In each ring, differentiation of secondary phloem and thick walled conducting elements of the secondary xylem was restricted to certain portions/segments of the vascular cambium while the rest of the segments/portions of the cambium exclusively differentiated into conjunctive tissue (Fig. 19C-E). Each newly originated cambium first gave rise to one to two parenchyma cells on either side to it. These parenchyma cells contributed to a large number of conjunctive tissues on both outer and inner side

of newly formed cambium. The parenchyma cells located outside to the newly formed cambium served as a site for the origin of future cambium. Very few cambial cells contributed to develop conjunctive tissue between previous cambium and newly developed cambium. As the secondary growth progressed further, more conjunctive tissues were added by the parenchyma cells present between two successive rings.

As mentioned earlier, 2-3 rings of cambia were active simultaneously (Figs. 19A-C), in which differentiation of xylem and phloem was relatively more extended and faster in the outermost ring while it was very slow and limited in the rest of the rings (Fig. 19D, E). As shown in figures 18D and 19A-C, all the cambia were functionally active in which the outer two rings have hardly produced any xylem derivative. As very few cells from the cambium differentiated into vessels to its inner side, thus they were mostly arranged in radial files (Fig. 19D, E).

3.4.1.2 *Diploclisia glaucanscens* (Bl.) Diels. and *Cocculus hirsutus* (L.) Diels.

***Diploclisia glaucanscens* (Bl.) Diels.** (Menispermiaceae)
Common Names: *Vatan-vel, Vatoli,* and *Batta-valli.*

***Cocculus hirsutus* (L.) Diels.** (Menispermaceae)
Common Names: *Ink Berry, Broom creeper, Vasanvel, Abashta* and *Dirghavali.*

Anatomy of young stems: Young stems of both *Diploclisia glaucanscens* and *Cocculus hirsutus* showed presence of one to two cell layered epidermis covered with trichomes (Figs. 21A, 22A). Cork was observed at maturity of the stem as protective layer (Figs. 21A, 22A). Epidermis was followed by 2-3 cell layered thin walled cholrenchymatous hypodermis. Cortex was formed beneath the hypodermis, which was composed of thin walled oval to polygonal parenchyma cells. Around 11-13 (in *Cocculus hirsutus*) and 8-12 (in *Diploclisia glaucanscens*) collateral vascular bundles were joined by interfascicular cambium to form a complete ring of the vascular cambium. Functionally, this cambium was bidirectional and produced secondary xylem toward inner side and secondary phloem toward outer side (Figs. 21A, 22A). Pericyclic fibres were formed near to primary phloem forming bundle cap of each vascular bundle (Figs. 21A, 22A). In both the species investigated, differentiation of such bundle caps was observed in 4-5 nodes away from the first visible internode of young branches. Pith was composed of parenchyma cells but as the secondary growth

progressed, some of the parenchyma cells were differentiated into sclereids such sclereids cells were observed in centre of pith of *Diploclisia glaucanscens* (Fig. 22E).

Structure and development of cambium: The mature stems of *Cocculus hirsutus* and *Diploclisia glaucanscens* were composed of successive rings of xylem alternating with the phloem (Figs. 20A-C, 22B). The cambium was non-storied with axially elongated fusiform cambial cells and horizontally arranged more or less isodiametric cuboidal clusters of ray cambial cells. The length of fusiform cambial cells was measured about 232-323 µm and 299-364 µm *Cocculus hirsutus* and *Diploclisia glaucanscens* respectively (Table. 5). The length of fusiform cambial cells was increased from the inner to the outer successive rings. During the early stages of stem development 11-13 (in *Cocculus hirsutus*) and 8-12 (in *Diploclisia glaucanscens*) collateral vascular bundles were joined by interfascicular cambium and formed a complete ring of cambium (Figs. 20A, 22A). This cambium remained functional for two to three years producing xylem to the inside and phloem towards the outside. Later on, it ceased to divide and a complete new ring of cambium was developed from the divisions of cortical parenchyma cells located outside the perivascular fibre bands (Figs. 21C-D, 22C-D). At the time of new cambium development, the inner cortical parenchyma cells underwent de-differentiation resulting in the formation of a group of radially arranged parenchyma cells. Some of the parenchyma cells towards the periphery of the stem differentiated into lignified parenchymatous conjunctive tissue while other parenchyma cells located beneath these underwent periclinal divisions forming a new cambial layer. Each successive ring of cambium developed in a similar fashion as described above.

In both the species, development of a new cambial ring occurred only after the cessation of cell divisions in the previous cambium. However, in some of the samples reactivation of the previous cambium was noticed at the time of development of a new cambial ring. Being a deciduous climber, fruit maturation and defoliation started in January-February and the plants remained leafless until May-June. The cambium remained dormant during this period and reactivation or development of new cambium occurred with the sprouting of new leaves in June.

Structure and development of vascular elements: Reactivation of the cambium resulted into bi-directional differentiation of xylem and phloem elements. After

lignification of these newly developed derivatives, it became very difficult to discern growth rings in axial xylem elements, but the radial system shows distended rays (also called ray nodding) at the ring boundaries. Ray nodding usually occurs in the region of resting cambial zone. In transverse view the nodes generally appeared spindle shaped and persisted distinctly in the xylem.

Each newly developing cambium first produced conjunctive tissue followed by lignified xylem derivatives. In these lignified elements, the first differentiating products were always fibre-tracheids, and development of vessels took place only after the production of 5-9 fibre-tracheids. In both, *Cocculus* and *Diploclisia*, with the increase in stem diameter, xylem ray width and height increased gradually from the centre towards the periphery. In *C. hirsutus*, ray height and width were maximal 6018 μm and 697 μm in the outermost ring and minimal 3818 μm and 365 μm in the innermost ring while in *D. glaucanscens*, it was maximal (1169 μm and 697 μm) in the outermost ring and minimal (988 μm and 365 μm) in the innermost ring respectively (Fig. 21E). Some of the ray cambial cells elongated vertically or underwent random elongation resulting in the development of deformed xylem fibres and vessel elements (Fig. 23A), while marginal ray cambial cells elongated radially and differentiated into libriform fibres (Fig. 23B-E). Ray parenchyma cells were thin-walled and showed a heavy accumulation of starch grains. Fibres in the rays which had a radial orientation also showed starch deposition. Conjunctive tissues produced in the beginning were found to be devoid of starch.

Similar to ray height, length of fibre-tracheid in both the species increased gradually in each xylem ring from pith to periphery and maximal length 1286 μm was observed in the outermost xylem ring and minimal length 850 μm in the innermost ring of *C. hirsutus* and it was 978 μm (maximal) and 750 μm (minimal) for *D. glaucanscens*. Similarly trend of increase in length of vessel element was also observed from the pith to the periphery in both the species investigated. In each successive ring of the xylem vessel lumen diameter decreased gradually and its frequency was increased from the centre towards periphery. Compared to fusiform cambial cells, length of vessel elements decreases slightly whereas width increases 6 to 8 times. Vessels were oval to circular and solitary, but radial multiples of 2 to 4 were also observed occasionally. They had simple perforation plates in slightly

oblique to transverse end walls. Vessel frequency and their lumen diameter showed a positive correlation (Table 4).

However, occurrence of tyloses in the vessels was common in all the xylem rings. Initially they were thin-walled, but in the course of time they underwent secondary wall deposition and become lignified. The pits on the sclerosed tyloses were oval-oblong and conspicuous due to lignification (Fig. 22F). At some of the places accumulation of starch was also observed in the tyloses.

The secondary phloem was composed of sieve elements, companion cells and axial and ray parenchyma cells. Sieve tube elements were the largest and companion cells were the smallest cells in transverse view while axial parenchyma cells were intermediate to them. Sieve tube elements possessed compound sieve plates with three to four sieve areas on their oblique end walls. The sieve tube elements adjacent to each cambial ring were functional with open sieve pores while the elements away from the cambial zone were non-functional and showed heavy accumulation of callose followed by obliteration of sieve tube members.

3.4.1.3 *Antigonon leptopus* Hook. & Arn. (Polygonaceae)
Common Names: *Coral vine, Mexican creeper* and *Love-vine.*

Anatomy of young stem: The young stem of *A. leptopus* was made up of single layer of thin walled more or less barrel shaped epidermal cells covered with glandular and uniseriate multicellular trichomes (Fig. 24D). Epidermis was followed by hypodermis consisting of 2-3 layered chlorenchyma cells while cortex was composed of parenchyma cells. Differentiation of pericyclic fibre bands was observed only after the fifth internode. At this stage, accumulation of tannins began in the cortical parenchyma and pith cells. Young stem was composed of 5-6 vascular bundles, which were joined by forming interfascicular cambium and completed the complete ring of cambium. This cambium was functionally bidirectional producing xylem centripetally and phloem centrifugally (Fig. 24D). Secondary growth leads to development of successive cambia (Figs. 24E, 25A).

Structure of cambium: The cambium was semi-storied with axially elongated fusiform cambial cells and horizontally arranged ray cambial cells. In transverse view, fusiform cambial cells of the well-developed vascular bundles appeared as

tangentially flattened radial rows of 3-5 cells while in developing bundles they were more or less isodiametric. Length and width of fusiform cambial cells ranged from 187-342 μm and 15-26 μm respectively (Table. 5). Cambial rays were uni-multiseriate, heterocellular and compound. Their height and width varied from 21-1800 μm and 18-340 μm respectively. Ray cambial cells appeared more or less isodiametric ranging from 16-23 μm in diameter.

Development of vascular cambium and its derivatives: During primary growth, the young stems were composed of five collateral vascular bundles. These bundles were connected by interfascicular cambium and formed a complete cambial ring giving a star shaped appearance (Fig. 24D). The cambium divided bidirectionally and produced xylem centripetally and phloem centrifugally. However, xylem and phloem development remained restricted only to the fascicular sector of the cambium whereas interfascicular region exclusively differentiated into parenchyma cells on both sides, giving an impression that vascular bundles are embedded in parenchymatous tissue (Fig. 24D). The first cambium remained functional for a definite period and ceased to divide towards xylem side. After the cessation of xylem development, a second ring of cambium originated from the parenchyma cells located outside to the primary phloem (Fig. 25D). Similar to the previous cambium, second ring of cambium also remained functional for a definite period and thereafter it ceased to divide. At this stage, the interfascicular cambium of previous ring differentiated completely into parenchyma cells while the fascicular regions maintained its radial arrangement (Fig. 25A, B). From the newly developed cambium, the first derivative to differentiate was the sieve element (Fig. 25E) followed by thick walled xylem derivatives. In these rings, some of the cambial segments exclusively differentiated into thick walled parenchyma and fibriform vessels (Fig. 25A, B). In such areas, wider vessels were found to be completely absent and if present, their frequency was much less (Fig. 25B). The tendency of the vessels to be absent in the early or later part of the secondary growth was seen frequently in most of the successive rings. At some places one or two vessels were formed immediately after formation of new ring or prior to the cessation of cambial growth while remaining part the xylem became vesselless. In some of the xylem rings vessels were either formed in the middle of the ring or entire ring became vesselless (Fig. 25B). Such pattern of vessel restriction was not observed in the secondary xylem produced by first ring of vascular cambium. The sieve tube

elements from the inner rings began to cease their function by extensive deposition of callose followed by obliteration of these sieve elements. These non-functional sieve tube elements were replaced by the newly formed sieve elements and axial parenchyma cells were arouse from the fascicular segment of the previous cambium.

Phloem remained functional in all the successive rings of vascular bundles owing to the replacement of non-functional phloem derivatives by addition of new elements. Compared with fusiform cambial cells, sieve elements were relatively shorter measuring from 148-258 μm in length. Sieve plates were simple on the slightly oblique to transverse end walls. Each sieve tube element is associated with a single companion cell.

Secondary xylem was composed of vessel elements, vascicentric tracheids, nucleated fibres, and axial ray parenchyma cells. Vessels were mostly solitary with simple perforation plate on the transverse end wall. Pits on the lateral wall were alternate, bordered and elliptic rather than round in which an alternate pattern intermixed with the scalariform pattern. Length and width of vessel elements varied from 73-165 μm and 80-265 μm respectively. On the basis of their diameter these vessels were classified into large and fibriform vessels. Wider vessel elements measured about 120-225 μm in length and 225-375 μm in diameter. Vessel lumen diameter, however, increased gradually from centre towards periphery in each vascular bundle (Fig. 25C). The fibriform vessels were like imperforate tracheary elements except for the occurrence of a small sub-terminal perforation plate near each end of the cell. They were measured about 210-425 μm in length and 37-55 μm in diameter. Xylem fibres were septate with slit like simple pits and 2-4 times (395-578 μm) longer than the fusiform cambial cells (Table. 5). Fibres also retained nuclei in each compartment even after the deposition of secondary wall. The nuclei were oval to oblong and fusiform shaped, measuring from 4-7 μm and appeared similar to the nuclei of axial ray parenchyma cells of the xylem. The parenchyma cells differed from xylem fibres by being broader, shorter and having thinner walls. They also possessed large circular, simple pits on both radial and tangential walls.

3.4.2 Successive Cambia Having Inverse Orientation:

3.4.2.1 *Dolichos lablab* L. (Fabaceae)

Common Names: *Hyacinth Bean, Indian bean, Valode* and *Vaal.*

Structure of cambium: The vascular cambium of *D. lablab* was storied with short, roughly hexagonal fusiform initials with abruptly tapered ends measuring about 210-245 mm in length, and cuboidal clusters of small ray initials. Cambial rays were mostly uni-multiseriate in that region of cambium where thick-walled xylem and phloem elements were produced, while other regions of cambium which was differentiating into thin walled conjunctive tissue on both xylem and phloem side were huge, multiseriate, compound, aggregate and polycentric. Polycentric rays were found where cambial ray cells with several initiation centers of small cells were formed by local frequent divisions. Height and width of these rays differed greatly and measured 65-4586 and 25-897 mm, respectively (Table. 6).

Development of vascular cambium and its derivatives: In the young stem, 12-15 collateral vascular bundles were joined by interfascicular cambium so that finally a complete ring of vascular cambium was formed. These cambial cells were functionally normal, producing thick-walled lignified xylem elements centripetally and phloem elements centrifugally. After the formation of a 3-4 mm thick band of xylem, the cambium ceased to produce thick-walled elements but development of parenchyma cells was continued. Several layers of parenchyma cells (approximately 2-3 mm) were originated from each radial file (Fig. 26A). Later the same cambium again produced thick-walled elements. The cambium thus behaves functionally like two distinct types: i) segments of cambium producing thick-walled secondary vascular tissue, and ii) segments of cambium producing only thin-walled parenchyma, both centrifugally and centripetally. The cambium that produced thick walled lignified vascular elements consisted of uni-triseriate rays while other segments that were producing parenchyma cells possessed multiseriate rays along with axial parenchyma. The height and width of these rays were ranged from 3893-4586 and 568-897 mm, respectively. New cambial segments were also observed developing from both axial and ray parenchyma.

The outermost phloem parenchyma produced by the previous cambium enlarged both in radial and tangential direction and underwent repeated divisions at several places, forming meristematic segments which developed into radially flattened cambial cells. These cells developed into xylem and phloem elements giving the appearance like vascular bundles. Subsequently, these cambial arcs were

interconnected by new cambial strips that developed from the parenchyma cells present between the bundles and formed a new ring of cambium. The cambial ring formed by this way produced conducting elements at certain portions of a cambial segment while the rest of the segments exclusively differentiate into parenchyma cells.

As the stem diameter increased further, the axial and ray parenchyma of the phloem underwent dilatation. At an early stage of dilatation, some part of the parenchyma became swollen followed by repeated cell divisions occurred which lead to the development of several small meristematic centers (Fig. 26D, E). Continuous divisions in these meristematic centers resulted in the development of vascular bundles. The dilatation also caused obliteration of adjoining cells. As shown in Fig. 26D, these bundles do not had a distinct orientation and they may be arranged radially, tangentially or diagonally. A similar pattern of ray and axial parenchyma dilatation followed by vascular bundle development was also observed in the secondary xylem. This led to a splitting of the xylem into small pockets embedded in thin-walled parenchyma (Fig. 28A-D). The xylem formed in the beginning of secondary growth was intact except at the regions of medullary rays (Fig. 27A, B). With the advancement of secondary growth, xylem underwent splitting making its structure quite complex (Fig. 27C, D). Development of vascular bundles among xylem rays was similar to the pattern described before.

Near ground level, *Dolichos* stems may be either vertical or horizontal in position. According to the position, the stem showed variation in the arrangement of successive rings of cambia (vascular bundles). In the vertically arranged stem, cambia were arranged in successive rings. Sometimes, the stem became lobed looking like "8" in transverse view due to the development of successive rings of vascular bundles on only on opposite sides (Fig. 27C). In horizontally growing stem, three-five successive rings of vascular bundles (cambia) occur eccentrically i.e. on the upper side while the lower portion possesses only one or two cambial rings.

Inverse orientation of vascular bundles: Vascular bundles, which had an inverse orientation (i.e. xylem outside and phloem inside), developed when the stem became 20-30 mm in diameter (Fig. 28A, C). A group of parenchyma cells situated deep inside the xylem then underwent repeated periclinal divisions and formed a wide band

of meristematic cells. These meristematic cells developed into small segments, which were distributed approximately at equal distance from each other. The cells in each segment of cambium divided periclinally and gave rise to phloem elements towards the centre of the stem and xylem towards the periphery (Fig. 28A, B). The xylem produced was composed of vessels, fibres and axial parenchyma cells, while the phloem was composed of sieve tube members, companion cells and axial parenchyma (Fig 28B). As the secondary growth progressed further, several such vascular bundles were formed (Fig. 28D). The length of vessel elements and sieve tube members remained more or less similar to that produced from the functionally normal cambium. Vessel members possessed simple perforation plates on their slightly oblique to transverse end walls. Pits on the lateral walls were alternate and bordered.

Structure of xylem: The secondary xylem was composed of vessels, tracheids, fibres and parenchyma while phloem islands were seen only in the innermost xylem. As the stem diameter increased, more and more parenchyma cells (both axial and ray) of xylem underwent dilatation followed by repeated divisions forming several vascular bundles. As shown in the schematic diagrams of the mature stem, the axial parenchyma cells present among xylem elements also underwent dilatation so that finally the xylem was dissected into small islands (Fig. 27D). The mature stem was composed of several vascular bundles embedded in the parenchymatous ground mass. These vascular bundles did not had any specific orientation, thus they appeared in radial or tangential orientation. Sometimes, two or more vessel elements were arranged end to end forming circular vessels. They may be small, formed of only two vessel elements, or larger with several vessel elements. Due to the dilatation, rays also lost their characteristic shape and became exceptionally large measuring about 3278-5874 mm in length and 840-3854 mm in width. Sometimes ray cells were differentiated into radially arranged vessel elements and fibres. These fibres were vertically elongated or arranged spirally. Some of the vessel members were also organized either tangentially or in zigzag manner.

3.4.2.2 *Ipomoea sp.* (following 3 species studied)

***Ipomoea hederifolia* L.** (Convolvulaceae)

Common Names: *Red Star Glory, Yellow Ivy Glory, Yellow Trumpet,Morning Glory.*

***Ipomoea biloba* Forsk.** (Convolvulaceae)

Common Names: *Samudraphens,* Beach *Morning Glory, Dopatilata Vine, Bamgvel.*

***Ipomoea quamoclit* L.** (Convolvulaceae)

Common Names: *Cypress Vine, Morning Glory, Cardinal Creeper, Ganesh vel.*

Morphology of the stem: Morphologically, the stem portion adjacent to the ground level and not in contact with any supporting object remained circular in outline and showed distinct concentric rings of cambia. However, the portion away from the ground and in contact with the supporting object showed differential cambial activity, ultimately resulting in a lobed pattern. The numbers of lobes were not constant and may vary for a given plant. The stem was 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. It has been observed that a lobed pattern formed as a result of increased cambial activity on the opposite side of the stem to the support. In *I. biloba*, and *I. quamoclit*, stem was mostly circular in outline but sometimes more or less angular in outline due to unequal activity of the cambium. The branched stem of *I. quamoclit* typically twines anticlockwise (rarely clockwise) around a support. The chlorophyllous stem was mainly cylindrical, irregularly-ridged and mottled reddish-purple at the ridges. The stem base was often deeply furrowed and flattened or broadly lobed and is reddish-brown or rarely yellow brown.

Anatomy of young stems: In the young stems of all three species i.e. *Ipomoea hederifolia*, *I. biloba*, and *I. quamoclit*, the epidermis was composed of single layered, compactly arranged isodiametric parenchyma cells. It was covered with a thin cuticle on the outer side while internally it contained 2-3 layered hypodermis comprising thin walled isodiametric parenchyma cells. The cortex was mainly composed of parenchyma cells along with several immature secretary ducts that were distributed randomly in it. In *I. hederifolia*, they were also developed in the pith simultaneously with the differentiation of internal phloem strands (Fig. 1A-F). However, the inner layer of cortex formed discontinuous bands of thick walled, lignified perivascular fibres. Based on the primary and secondary growth, developmental anatomy of all the three species of *Ipomoea* may be classified into five stages: two in primary growth, (a) the bicollateral bundle stage and (b) the cambium like meristem stage and three of

secondary growth consists of (c) normal cambial stage, (d) the anomalous (reverse orientation of cambium) cambial stage and (e) supernumerary cambial stage.

Development of vascular cambium: A normal vascular cambium was originated as a continuous cylinder between the external protophloem and metaxylem. The cambium functions in a normal way as other dicots for a short time and produced a few layers of secondary phloem centrifugally and secondary xylem centripetally. The secondary phloem consists mainly of sieve tubes, companion cells and phloem parenchyma cells while secondary xylem consists of vessels, fibriform vessels, fibre-tracheids and xylem ray parenchyma cells. This cambium remains functional for definite period producing 5-6 mm of secondary xylem, which later ceased to divide. Up to this stage, the protoxylem remained unchanged while clusters of internal phloem increases slightly in size due to the differentiation of additional pith cells into sieve tube elements. The cortex consists of discontinuous bands of perivascular fibres, large thin walled parenchyma cells, secretary structures and druses containing cells.

A second ring of cambium developed from the axial parenchyma cells at a distance of about three to six cell layers outside the phloem produced by the previous cambium. During the development of new cambium, one or two parenchyma layers underwent repeated divisions and resulted in the formation of five to six layered wide bands of meristematic cells. Initially these cells were arranged irregularly but further periclinal divisions in them leads to radial arrangement (Fig. 29A, B). Cells in the centre of this band differentiated into vascular cambium while the remaining cells on either side of newly formed cambium differentiated into conjunctive tissues centripetally and outer cells into secondary cortex. However, these conjunctive tissues formed the site for the origin of inverse cambium (Fig. 29C, D). The newly developed cambium from the axial parenchyma was functionally normal producing secondary xylem towards the centre and secondary phloem towards periphery (Fig. 29E, F). Each functionally normal successive cambia followed similar pattern of its development. The first ring of cambium only forms the continuous band of xylem while the second ring onwards differentiation of conducting elements of both xylem and phloem remained restricted to certain segments of the meristem while rest of the portion forms thin walled conjunctive tissue on both outer and inner side of it.

The mature stem was composed of 5-6 successive rings of xylem alternating with phloem (Figs. 30A, 31A). Among these five rings of secondary xylem were produced from the functionally normal cambium. In most of the rings there remained another discontinuous ring of secondary xylem, which was produced by functionally inverse cambium i.e. secondary xylem arranged centrifugally and phloem centripetally (Figs. 30A, B, 31A-C). However, these cambia do not form a complete ring, rather it is in the form of small segments present only on the lateral side of the flat stem. In some of the stem segments, tangentially arranged bicollateral vascular bundles are also observed.

Structure of secondary xylem: The xylem was diffuse porous with indistinct growth rings and composed of both wider vessel elements as well as very narrow fibriform vessels, which are indistinguishable in transverse view. Vessels were mostly solitary with simple perforation plate on their slightly oblique to transverse end walls. Their length and width measurement ranged from 125 to 476 μm and 134 to 325 μm respectively in all species studied. The alternate bordered pits on the lateral walls are oval to elliptic and oblong with 7-10 μm in diameter. Tyloses are common in the wider vessels in all the successive rings and they are mostly thin walled.

Like other members of the Convolvulaceae, occurrence of fibriform vessels are also recorded in *I. hederifolia*. Fibriform vessels were like imperforate tracheary elements except for the occurrence of a small sub-terminal perforation plate near each end of the cell. It is difficult to distinguish their fibriform vessels in transverse view and their dimensional details were studied either in longitudinal sections or in the macerated material. The length and width of fibriform vessel elements varied from 478-518 μm and 28-34 μm respectively. Xylem rays were mostly uniseriate but biseriate rays were also frequent. Ray cell walls were thick and lignified measuring about 1.2-1.4 μm. Conjunctive tissues are mostly thin walled and unlignified parenchyma that forms a band of 6-10 cells in each radial file and serves as a site for the origin of reverse cambium.

Origin of functionally inverse cambia: The second ring of cambium was functionally bidirectional, as in other dicotyledons that form successive cambia. After the formation of 15-20 xylem elements by this cambium, the parenchyma (conjunctive tissue) adjacent to the inner side of xylem derivatives underwent

periclinal divisions and resulted in the development of a cambial zone of three to four cells wide (Figs. 29A, B, 30C). This cambium was functionally abnormal (inverse), producing secondary xylem towards the periphery and phloem towards the centre (Figs. 29D, 30D, 31B, C, E). Before the cessation of cambial cell division in the second normal cambium, the inverse cambium produced around 20-25 xylem derivatives and 8-10 phloem elements. Development of further inverse cambia followed a similar pattern. Structurally, considerable variations were also observed in the secondary xylem of functionally inverse cambia. It was mostly composed of narrow fibriform vessels, fibre-tracheids, and axial and ray parenchyma cells. Wide vessels were rare or absent. They were mostly solitary with a simple perforation plate on their slightly oblique to transverse end walls. In comparison with normal secondary xylem and phloem, its amount was relatively less, which is measured about 6-9 mm and 3-5 mm respectively.

3.5 INTERXYLARY PHLOEM PRODUCED BY A SINGLE CAMBIUM

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 as in *Calycopteris*, *Combretum*, *Leptadenia reticulata* and in several species of *Strychnos* (Mikesell and Popham 1976; Carlquist 1988; Patil and Rajput 2008). Den Outer and Van Veenendaal (1995) recommended that scattered, isolated strands of secondary phloem embedded in secondary xylem should be named *diffuse included phloem* instead of interxylary or included phloem of the foraminate type (IAWA Committee 1989).

Carlquist (1988) stated that intraxylary phloem may be a sort of precursor to interxylary phloem, for example, the order Myrtales characteristically has intraxylary phloem, but occurrence of interxylary phloem is scattered in the species of Myrtalian families. 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 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 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 the present study development of included phloem and its structure was studied in *Leptadenia reticulate*, *Salvadora persica*, *Coccinia indica*, *Strychnos bicolor*, *Ipomoea hederifolia*, *Canavalia ensiformis*, *Mucuna pruriens* and *Calycopteris floribunda*.

3.5.1 *Combretum* Type:

Combretum subtype, according to Eames and McDaniels (1947) in generas 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.

3.5.1.1 *Leptadenia reticulata* (Retz.) W. & A. (Asclepiadaceae)

Common Names: *Dodi, Shingutti, Hirandodi and Kharkhoda.*

Development of Included phloem and Secondary growth: Like other dicotyledons, with the initiation of secondary growth, the cambium produced secondary xylem centripetally and secondary phloem centrifugally. After the formation of 25-30 elements of the secondary xylem, certain segments of the vascular cambium temporarily ceased to produce secondary xylem derivatives internally and began to reproduce sieve tube elements and axial parenchyma cells internally (Fig. 32A). During differentiation, there was no direct differentiation of phloem elements rather the cambium formed parenchyma cells along with isolated phloem mother cells embedding in it. These phloem mother cells later on differentiated into sieve tube elements. However, direct differentiation of sieve tube elements on the inner side of the cambium was observed occasionally (Fig. 32B, C). It seems that when the cambial cells differentiate into axial parenchyma, some of the cambial cells (residual meristem) also get shifted towards the secondary xylem, which later on differentiates into sieve tube elements. However, in transverse section these residual cambial cells look like parenchyma cells and are thus indistinguishable from the surrounding parenchyma cells.

After a short period of such activity (i.e. after the formation of 4-8 parenchyma cells/sieve tube elements) these cambial segments regained normal cambial activity and began to produce thick walled secondary xylem centripetally. Later, another segment of the cambium behaves in a similar manner. Such repeated behavior of the cambium led to the formation of phloem islands along with parenchyma embedded in the secondary xylem, resulting in included phloem (Fig. 32A).

As the stem thickness increased, segments of cambium producing parenchyma cells and sieve tube elements also became wider and formed larger bands of included phloem embedded within xylem. Sometimes these bands covered three to four adjacent rays, therefore, some of the rays passed through the phloem islands (Fig. 32D-F). In mature stems (i.e. 15 to 20 mm diameter), the sieve tube elements produced in the beginning of secondary growth became non-functional by heavy accumulation of callose. The non-functional sieve tube elements gradually collapsed and crushed completely, leading to radial and tangential expansion of the adjacent parenchyma cells. Later on these parenchyma cells underwent periclinal divisions and differentiated into new sieve tube elements (Fig. 32F). Structurally the sieve tube

elements of both normal and included phloem were more or less similar. The phloem was composed of sieve tube elements, companion cells and axial parenchyma cells, which were always found in contact with the adjacent ray cells (Fig. 32D, E). Both the types of phloem differentiated in the stem differ in the length and width of the sieve tube elements. The sieve tube elements were longer and wider in included phloem (238 μm and 22 μm) than in external phloem (209 μm and 20 μm) (Table. 7).

3.5.1.2 *Salvadora persica* L. (Salvadoraceae)

Common Names: *Toothbrush* tree, *Mustard* tree, *Arak* tree, *Peelu* tree and *Miswak.*

Anatomy of young stem: In young stems of *Salvadora*, a single layered epidermis was covered with thin cuticle. The epidermal cells were compactly arranged and more or less barrel shaped. With the increase in stem diameter, the epidermis was replaced by a superficial layer of cork, whose outer cells flake off at intervals. A 2-3 layered hypodermis located beneath the epidermis was composed of oval to polygonal cells containing chloroplast. The cortex was initially broad in young stage, but later on it became narrow due to increase in pressure of newly added secondary xylem. The thin walled cortical cells often found to contain druses or rhomboidal crystals. Pith parenchyma cells were pitted and arranged loosely showing intercellular spaces. Some of the pith cells also contained solitary druses.

At the time of secondary growth, several collateral vascular bundles were joined by interfascicular cambium and formed a complete cambial cylinder. Functionally this cambium was bidirectional and gave rise to secondary xylem centripetally and secondary phloem centrifugally.

Structure of cambium: The cambium was storied and composed of axially elongated fusiform initials and isodiametric ray cambial cells. Fusiform cambial cells were arranged in the storied manner and were roughly hexagonal while cambial rays were uni-multiseriate, compound and heterocellular with marginal sheath cells (Fig. 33B). Fusiform cambial cells were measured from 218-298 μm in length and 24-28 μm in width while ray cambial cells were 23-34 μm in diameter. During dormant condition, the radial walls of the fusiform cambial cells were beaded due to the presence of numerous primary pit-fields.

Development and structure of included phloem: After a period of primary growth and the formation of first ring (normal) of cambium, bidirectional differentiation of vascular elements occurred for a short period. Soon after the initiation of secondary growth, cambial behavior became unusual and began to produce secondary phloem on either side of the cambium. Differentiation of this interxylary phloem took place in two ways: i) direct differentiation of cambial derivatives into sieve tube elements in association with parenchyma cells and ii) delayed development of sieve tubes from the re-differentiation of xylem parenchyma. In the former type, cambial derivative cell underwent periclinal division and formed one smaller cell and other with relatively wider diameter. From these, smaller cell differentiated into companion cell while larger cell formed sieve tube member. Therefore, at certain places phloem cells may be seen on both sides of the cambium cylinder. In the latter type of phloem differentiation, the outer margin of the secondary xylem became more or less indented in outline in the young stems (about 3-4 mm in diameter) and the depressions were filled with thin walled tissues. It is due to the fact that at certain places, the cambium cuts off either secondary phloem elements (sieve tube elements) or thin walled tangentially flattened groups of parenchyma in place of usual lignified wood cells (Fig. 33C). These cells lie in perfect radial rows and differ from the cambial cells by their relatively large in diameter (Fig. 33C). This is however only temporary phase and after a short period of cell division activity, the cambium returned to its normal activity so that quest of thin walled parenchyma became enclosed between the thick walled cells of the previous and the newly formed wood (Fig. 33E). As growth progressed further, the central cells in the islands differentiated into one or more groups of sieve tubes elements and companion cells but the peripheral cells still remained parenchymatous (Fig. 33D, E). These peripheral cells often underwent some periclinal divisions and formed a weak secondary cambium on the inner as well as the outer face of the phloem island. In the older and consequently deeper seated phloem islands some the central cells underwent degeneration, thus forming darkly staining patches of obliterated phloem in each island (Fig. 33A, D).

Structure of vascular elements: The xylem was diffuse porous with indistinct growth rings and composed of vessel (both narrow and wider), fibres, rays and wide tangential bands of axial parenchyma cells. Vessels were solitary but usually arranged in radial multiples of 2-4 vessel elements while narrow vessels were mostly solitary

with simple perforation on slightly oblique end walls. The average length and width of large vessel elements was ranged from 212-312 μm and 250-360 μm respectively while medium vessels were 187-237 μm length, 137-200 μm width. Rays were mostly uni-biseriate; whereas multiseriate rays were observed occasionally. They were composed of procumbent, square, and upright cells in about equal numbers. Occurrence of starch grains in ray cells was a common feature while rhomboidal crystals were recorded occasionally. The phloem formed from the cambium on external side mostly comprised of parenchyma cells while sieve tube elements and companion cells were relatively less. Sieve tube elements possessed simple sieve plate on its' obliquely end walls. Length and width of sieve tube elements was measured about 245-253 μm and 27-33 μm respectively. No much structural variations were observed between the outer normal phloem and the interxylary phloem except in case of former one, isolated or groups of 2-3 sclerenchyma cells were distributed irregularly.

3.5.1.3 *Coccinia indica* L. (Cucurbitaceae)

Common Names: *Ivy Gourd, Kundru, Bimbi, Tundika, Ghiloda, Tondali* and *Tindora.*

Anatomy of young stem: In the young stems, the epidermis was made up of single layer of compactly arranged isodiametric and thin walled parenchyma cells. A thin layer of cuticle covered the epidermis while 2-3 layered hypodermis composed of parenchyma cells differentiated beneath it. The bulk of cortex showed heavy accumulation of circular starch grains. Endodermis and pericycle were not distinct. In the young stems, developments of five major bicolateral vascular bundles were observed from the promeristem giving penatngular shape to the young stem.

Structure of secondary xylem: In *Coccinia indica,* secondary growth in the main stem was achieved by the normal activity of the vascular cambium that formed secondary xylem centripetally and secondary phloem centrifugally. In the 6-8 years old plants, secondary xylem was diffuse porous with indistinct growth rings with large and heterocellular rays (Fig. 34A, B). The stem was mainly composed of parenchymatous ground tissue, in which thick-walled lignified elements of xylem were embedded as bands. These radial xylem bands were composed of vessels, fibres, and narrow sheaths of vasicentric axial parenchyma with some some apotracheal

parenchyma cells. In transverse view, xylem bands were frequently separated by large rays. These wide rays often developed into polycentric rays i.e. ray cells dedifferentiate into meristematic centers at several places and developed into bicollateral vascular bundles (Fig. 34C) with one or two vessels only. Very wide multiseriate rays were 985 to 1478 μm in height and 18-55 cells wide. In tangential view, they were heterocellular and most of the ray cells were procumbent, though square and upright cells were especially found on the ray periphery. Ray cells were 22.8 to 29.6 μm in diameter and no uniseriate rays or uniseriate wings were observed on multiseriate rays. In the mature stems, parenchyma cells surrounding the inner phloem of the bicollateral vascular bundles re-differentiated into meristematic cells and formed a ring with cells arranged in radial files appearing like a fully closed cambium (Fig. 34D).

Occurrence of fibre dimorphism was also observed in *Coccinia indica*: they septate libriform fibres with small slit-like pits (4.5-5.0 μm) at narrow angles with the main axis (4.5 to 5 μm) co-occurred with non-septate fibres with relatively large simple pits (with apertures of 3.3 to 4 μm) and wider lumen, giving them a parenchyma-like appearance. Non-septate fibres were 687 to 1125 μm long and 23.7 to 29.3 μm in diameter; septate fibres were 1057 to 1279 μm long and 20.8 to 24.6 μm in diameter. The non-septate fibres formed the bulk of cells in which septate fibres were intermingled. It was possible to distinguish both types of fibres in macerated material and tangential or radial longitudinal sections but in transverse view they were very difficult to distinguish (Fig. 35A).

Vessels were mostly solitary but radial or tangential multiples of 3-5 vessels also occured. Usually vessels were oval to oblong and often obstructed by numerous tyloses (Fig. 35B), which sometimes completely blocked the lumen. Such tyloses often contained small starch granules. Vessel elements were 186to 269 μm long and 250 to 385 μm wide and possessed simple perforation plates on slightly oblique to transverse end walls (Table. 7). The vessel-parenchyma pits on the lateral walls show pseudo-vestured and opposite to alternate with reduced borders, measuring 6 to 10 μm in horizontal diameter and 5 to 7 in vertical diameter (Fig. 35C-E).

Development of interxylary and secondary intraxylary phloem: Interxylary phloem development from the axial and ray parenchyma cells of the secondary xylem

were shown in 6-8 years old, mature stems (Fig. 36A-E). However, development of interxylary phloem from these parenchyma cells may be categorised into three distinct types: i) de-differentiation of axial parenchyma cells into meristematic cells forming concentric rings/circular cambia around intraxylary phloem (Fig. 36A, B), ii) de-differentiation of axial parenchyma into interxylary meristem that undergo differentiation into sieve tube elements (Figs. 36C-E, 35A) and iii) de-differentiation of xylem ray cells into meristematic regions and re-differentiation into sieve tube elements (Fig. 37B-E).

In the first type, initially some of the axial parenchyma dedifferentiated into meristematic regions and re-differentiated into isolated strands of interxylary phloem. As the sieve tube elements became non-functional, they collapsed and underwent obliteration. That resulted in radial and tangential expansion of surrounding cells followed by tangential divisions in them (Fig. 36A, B). These strands of interxylary phloem were much more distinct only after the cessation of function by more and more sieve tube elements. Therefore, collapse of the central cells and periclinal divisions in the surrounding cells resulted in the development of several meristematic centers throughout the stem (Fig. 36B). Cells in these meristematic centers differentiated only into the sieve tube elements while formation of xylem derivatives was not observed in any of the samples studied.

In the second type, unlignified axial parenchyma cells around the vessel elements de-differentiated into meristem and re-differentiated into interxylary phloem (Fig. 34C). In this type, the periclinal divisions were arranged in radial files like vascular cambium (Fig. 36D, E). Cell division and differentiation remained unidirectional thus producing only sieve tube elements and no xylem derivatives. Unlike in the former type, no circular/concentric meristematic rings are formed. The meristematic cells divide irregularly in various orientations. Thus, the meristems may be radially, tangentially or diagonally arranged (Figs. 35C-E, 36A). Sometimes the axial parenchyma between two adjacent vessels dedifferentiated into the meristems (Fig. 37A). Therefore, the meristematic regions located opposite each other produce more and more phloem derivatives which ultimately met in between these meristematic zones (Fig. 37A).

In the third type, ray cambium was formed by radial or tangential divisions in the unlignified marginal ray parenchyma cells (Fig. 37B). Prior to the development of secondary phloem, marginal ray cells of the xylem situated deep inside the mature stem underwent radial or tangential divisions to form 2-3 layers of meristematic cells arranged in radial files like the vascular cambium (Fig. 37C, D). These cells underwent further divisions and began to differentiate into interxylary phloem. However, divisions in these cells were not synchronized; the origin of phloem from ray cambium differed in different samples or sometimes within the same sample studied. Thus, development of phloem from the ray cambium may be further categorized into two types: i) differentiation of interxylary phloem towards the xylem side (Fig. 37C) consequently resulting into accumulation of phloem between thick-walled xylem derivatives and xylem rays (Fig. 37D) and ii) differentiation of interxylary phloem in the middle of meristematic cells result into differentiation of more and more sieve tube elements (Fig. 37E). Presumably, development of new phloem on either side of the ray cambium always exerted a pressure on earlier formed phloem that ultimately led to the crushing and obliteration of nonfunctional sieve tube elements in the middle of the meristematic band (Fig. 37B, E).

As the sieve tube elements become nonfunctional, it loses its turgidity, begins to collapse and is obliterated. The nonfunctional sieve tube elements were characterized by accumulation of callose and complete sealing of the sieve pores. Unlike in other dicotyledons, the callose was absent from the sieve plates which were completely obliterated (Fig. 37F).

Paedomorphic features of the xylem: Secondary xylem was arranged in plates representing an extension of the primary vascular system. Parenchyma cells were abundant, unlignified and apotracheal. They were arranged in the form of bands or variously shaped patches between the adjaçent vessels (Fig. 34A). Rays were multiseriate, and in addition to the development of new rays in the fascicular region of the cambium, the interfascicular region of the primary xylem also continued into very high rays of the secondary xylem (Fig. 38A). Ray cells were thin-walled, unlignified and predominantly upright (Fig.38B), few of them were square while procumbent cells were observed in the middle of the rays in radial longitudinal view. The shape and arrangement of lateral wall pits was altered somewhat by the presence of wide pit

aperture which were interconnected by grooves or coalescent pit apertures. Such pits are also referred to as unilaterally compound pits (Fig. 38C, D).

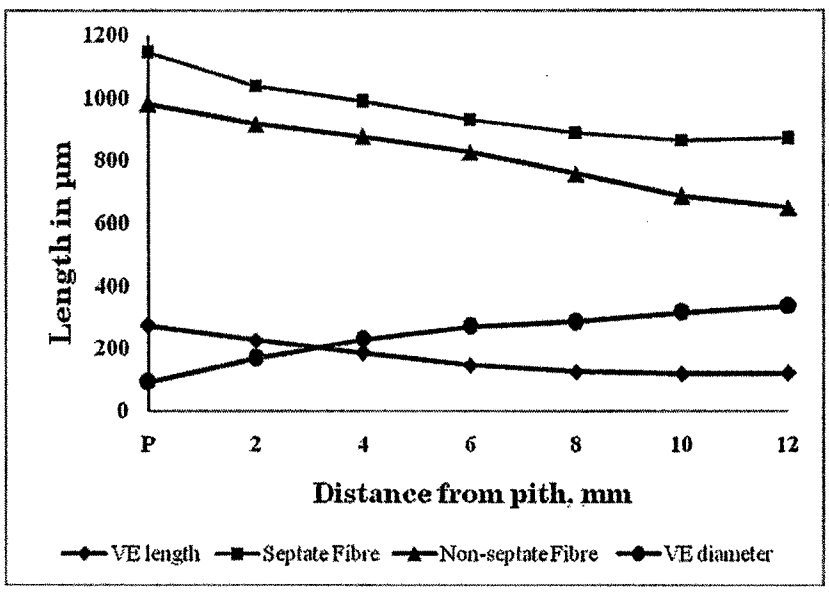

Figure VII: Length-on-age curves for vessel elements, septate and non-septate fibres in *Coccinia indica.* (VE - Vessel Elements)

The length-on-age curve of the vessel elements showed an overall negative slope i.e. vessel element length decreases from pith towards the periphery (Fig. VII, 38E). Similar kind of slopes were observed for the length of both septate (narrow) and non-septate (wider) fibres (Fig. 38F).

3.5.2 Scattered or Diffused Type or *Azima* Type:

In *Azima* subtype certain groups of parenchyma cells of the secondary xylem divide (dedifferentiate) and re-differentiate into phloem elements

3.5.2.1 *Ipomoea hederifolia* L. (Convolvulaceae)

Common Names: *Red Star Glory, Yellow Trumpet Morning Glory.*

Anatomy of young stem and development of successive cambia: Anatomy of young stem and development of successive cambia is described in primary unusual growth (Please see page Nos. 30, 60 and 66).

Development of the interxylary phloem: The phloem islands were not arranged in regular order; rather they were discrete and irregularly distributed in the xylem. They were smaller and fewer or almost nil towards the xylem formed at the periphery (Fig. 39E, F), but they were considerably larger and more numerous in the inner and older portions of the stem. This was so because the phloem was not directly differentiated from the cambium but it was rather developed from the re-differentiation of axial parenchyma after certain age. Development of included phloem began first in the parenchyma cells of the innermost ring of xylem formed by first ring of the cambium and then extended gradually towards the periphery. As mentioned earlier that *I. hederifolia* possessed both, thin walled as well thick walled lignified parenchyma (Figs. 39C, D, 40A, B). Differentiation of included phloem was noticed first in the innermost thin walled parenchyma (Fig. 39E). Prior to the development of sieve tube elements, these parenchyma cells underwent radial and tangential swelling followed by repeated periclinal divisions (Fig. 39E). Daughter cells formed after repeated divisions differentiated into included phloem elements.

Parenchyma cells formed in the beginning of secondary growth were always thick walled (Fig. 40A, B) therefore, differentiation of included phloem from these cells was seen only when stems were more than one centimeter thick. Prior to the development of included phloem, the thick walled parenchyma cells formed immediately after the initiation of secondary growth underwent de-differentiation and became thin walled (Fig. 40C-F). During de-differentiation, all these thick walled parenchyma lost their thickening by the loosening of the cell walls; whereas tracheids and narrow lumen vessels were the only elements which maintained their thick walls (Fig. 40E). Prior to the periclinal divisions these parenchyma underwent radial and tangential expansion (Fig. 40E-F) and formed a group of meristematic cells (Fig. 40F) which were arranged in radial file giving an appearance like a cambial zone (Fig. 40F). Cells located in the middle of these meristematic cells differentiated further into sieve tube elements (Fig. 40F). Structurally, this phloem differed from the normal phloem formed by the cambium in having more or less transversely arranged simple sieve plates. Sieve tubes with obliquely arranged sieve plates were also observed occasionally (Fig. 40B, C).

They also showed presence lateral sieve areas on their radial walls (Fig. 40D). However, there was no consistency in the length of the sieve elements (151 to 350 μm) as compared to that of normal phloem (323 to 368 μm) produced by the cambium. This is so because the parenchyma that differentiated into sieve elements may be divided transversely. In the rays of secondary phloem, radially arranged sieve tube elements were also noticed either solitary or in groups of two to four. When compared with ray parenchyma cells these elements were more or less similar in length while slightly larger in diameter and possessed simple plate on their transverse to slightly oblique end walls. Like axial sieve elements, each ray sieve elements was associated with single companion cell at their corners. These sieve elements were very well developed like axial sieve elements and possessed simple sieve plate with poorly developed lateral sieve areas. Most of them were shorter than their width, measuring about 23-32 μm in length and 28-38 μm in width but relatively longer than their width were also observed very often in all the samples studied. These sieve tube elements were observed in both uniseriate as well as multiseriate rays and always found at the margin of rays. Although, they were developed from marginal ray cells they were always observed in contact with the axial sieve elements in all three planes.

3.5.2.2 *Canavalia ensiformis* (L) DC. (Fabaceae)

Common Names: *Jackbean, Horse Bean, Sword bean, Tamateballi* and *Abai.*

Structure of Cambium: In the young stems, cambium was storied with vertically elongated fusiform initials and horizontally arranged isodiametric, cuboidal clusters of ray initials. Cambial rays were mostly multiseriate, compound (tall) and heterocellular (Fig. 41B) but unicellular rays were also seen occasionally. The length and width of the fusiform cambial cells varied from 288-310 μm and 13-24 μm (Table. 7), respectively. The cambial rays were uni-multiseriate and 140-584 μm in height and 22-450 μm in width.

Structure of xylem: Secondary xylem was diffuse-porous with indistinct growth rings. It was composed of fibres, vessels, included phloem and unlignified axial and ray parenchyma cells. These parenchyma cells formed the ground tissue of the stem in which lignified elements (xylem fibres and vessels) were present in pockets (Fig. 41A). These lignified elements ranged from small pockets solely of vessels surrounded by strengthening fibres or islands of fibres. The non septate fibres with

simple pits varied in their length from 1800-2500 μm. The vessels were dimorphic in nature with exceptionally large (348-389 μm) and small (85-138 μm) lumen diameter while some of them were intermediate (158-248 μm) in size. The length of the larger vessel elements always remained shorter than its width. The average length of the vessel elements varied from 68-271μm and diameter varied from 150-300 μm. Perforation plates were simple on their slightly oblique end walls to transverse end walls. Vessels were mostly solitary but radial multiples of 2-3 vessels were also observed occasionally.

Structure and development of included phloem: Development of included phloem was delayed and it was observed only after the formation of 4-5 mm of secondary xylem by the cambium while no sieve tube elements were observed in recently formed xylem derivatives as well as near the cambium. Some of the xylem parenchyma situated deep inside (2-3 mm away from the cambium) the secondary xylem underwent periclinal divisions resulting into small and large cells. Among them one of the cells (particularly larger one) differentiated into a sieve tube member and the smaller one into a companion cell. Thus, the islands of included phloem were always either in the form of isolated sieve elements or in a group of 2-3 sieve tube members (Fig. 41C-E).

In all the samples studied, isolated or a groups of 2-3 sieve elements were observed intermixed with the axial parenchyma of the xylem (Fig. 41C-E). These sieve tube elements were characterized by presence of simple sieve plates with well-developed sieve areas on their lateral walls (Fig. 41C, E). Each sieve element was accompanied by a single companion cell. The length of the sieve tube elements was measured from 270-295 μm. Functional sieve elements possessed slime (P-protein) plugs, starch grains and deposition of callose around the sieve areas while non-functional sieve tube elements showed heavy accumulation of callose and collapse of the sieve tube members.

Included phloem islands began to cease its function by loses of cell content and heavy accumulation of callose followed by obliteration of sieve elements. With the initiation of obliteration, adjacent parenchyma of the phloem islands enlarged in both radial and tangential direction and underwent periclinal divisions. This resulted

in the development of additional parenchyma, which compensates the space formed by obliteration of non-functional sieve elements.

3.5.2.3 *Mucuna pruriens var. pruriens* (Linn.) DC. (Fabaceae)

Common Names: *Velvet bean, Cowitch, Kiwanch, Kapikacchu,* and *Khaajkuiri.*

Structure of cambium: In both stem and root, the cambium was storied with vertically elongated fusiform initials and more or less isodiametric, ray initials. Cambial rays were uni-multiseriate, compound and heterocellular. Occasionally unicellular rays were also observed. The length and width of the fusiform cambial cells measured from 258-343 μm and 18-24 μm, respectively. The cambial rays were uni-multiseriate and 240-584 μm in height and 32-50 μm in width.

Structure of xylem: Secondary xylem was diffuse-porous with indistinct growth rings and was composed of fibres, vessels, and interxylary (included) phloem, and unlignified axial and ray parenchyma cells. Parenchyma cells formed the ground tissue of the stem in which lignified elements (xylem fibres and vessels) were embedded in pockets (Fig.42A, B). These lignified elements ranged from small pockets solely of vessels surrounded by strengthening fibres or only islands of xylem fibres. The non septated fibres with simple pits vary in their length from 1062-1750 μm. Vessels were mostly solitary but radial and tangential multiples of 2-3 vessels were also observed. Vessels may be categorised into vessels with large and narrow diameter. The narrow vessels were distinct and always surrounding the large vessels (Fig. 42A, B). Diameter of the large vessels elements was measured about 350-500 μm while narrow vessels were 150-275 μm. The length of the larger vessel elements was shorter than its width. Perforation plates were simple on the transverse to slightly oblique end walls to transverse end walls.

Structure and development of included phloem: In thick roots (12-15mm), pockets of axial parenchyma cells situated deep inside the secondary xylem underwent de-differentiation and acquired meristematic character. From these, individual cells divided periclinally and formed two unequal daughter cells which re-differentiated into companion cell and sieve tube element (Fig. 42B, C). As the individual cells from the parenchyma pockets acquired meristematic character, therefore the islands of included phloem were always in a group of 2-3 sieve tube members (Fig.42B-D).

Sometimes, differentiation of interxylary phloem was also observed from the parenchyma cells located between adjacent vessels (Fig. 42C). Sieve tube elements were characterized by presence of simple sieve plates with well-developed sieve areas on their lateral walls (Fig. 42D). Each sieve tube element was accompanied by a single companion cell (Fig. 42B-D). The length of the sieve tube elements was measured from 218 to 280 μm (Table. 7). As compared to external normal phloem, the length of the included sieve tube elements was always found to be less.

3.5.3 *Strychnos* Type:

In this 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

3.5.3.1 *Strychnos bicolor* Prog. (Loganiaceae)

Anatomy of young stem: Anatomy of young stem of *Strychnos bicolor* is described in primary unusual growth (Please see page No. 39 along with development of intraxylary phloem).

Structure of secondary xylem: Secondary xylem of Strychnos was diffuse porous with indistinct growth rings (Fig. 10A). Several irregularly arranged phloem islands of various shapes and dimensions were embedded in thick walled lignified xylem cells (Fig. 10A). It was composed of tracheids, vessels (both wider and narrow vessels), fibres with bordered pits, axial and ray parenchyma cells while fibriform vessels were not seen in any of the samples investigated.

Wider vessels were mostly solitary and possessed simple perforation plate on its oblique end walls. They were 273-571 μm in length and 79-176 μm in tangential diameter. On the other hand, narrow vessels were relatively larger than the fibriform vessels as reported for other scandent species. They were relatively longer than the wider vessel element with 293-588 μm in length and 28-63 μm in tangential diameter. The xylem fibres were characterised by the presence of bordered pits (Fig. 10E, F), thick lignified walls, narrow lumen and tapering ends. The bordered pits were oval to circular in outline measuring about 4.3-6.2 μm in tangential diameter with very distinct margins. The pit apertures formed a narrow angle with the fibre axis. Fibres

were 739-1200 μm in length and 7-16 μm in width. Occurrence of pith flecks was a common feature in all the samples investigated (Fig. 10C, D). In transverse view, they appear as conspicuous irregular islands of larger parenchyma cells with different dimensions and shape (Fig. 10C, D) ranging from 500 μm to 3000 μm in tangential and 95 μm to 2785 μm in radial plane.

Rays were mostly uniseriate but 2-3 seriate rays were also observed frequently. They were heterocellular, in which terminal ray cells were procumbent and vertically elongated while rest of them were more or less isodiametric. They were thick and lignified in the xylem region while unlignified and thin walled when traversing through the interxylary phloem island with relatively large, oval to circular simple pits.

Development of interxylary phloem: Initially the cambium formed secondary xylem internally and secondary phloem externally. In small segments of the cambium, inward development of secondary xylem was retarded and gradually stopped (Fig. 11A) while outward development of secondary phloem continued. Thus, the vascular cambium formed a depression in the areas where formation of xylem was stopped (Fig. 11A). Development of these depressions resulted in detachment of such segments from rest of the cambial cylinder (Figs. 10B, 11B). New cambial cells were originated on outer margin from the phloem parenchyma cells located outside to these segments (Fig. 11C-F). These newly formed cambial cells joined with the detached ends and completed the cambial cylinder (Fig. 43A). The cambial cylinder continued dividing bidirectionally producing secondary xylem internally and phloem externally. Formation of each new segment followed similar pattern thus leading to complete embedding of the sunken segments of the cambium that ceased temporarily to divide internally. The embedded cambial segments of each phloem island remained active forming secondary phloem externally while differentiation of xylem cells was very slow. Sieve tubes were rarely formed in the external secondary phloem therefore; most of the phloem elements were developed in the form of interxylary islands (Fig. 11B, D).

Phloem formed by the external cambium cylinder was mostly composed of parenchyma cells that contain rhomboidal to rod shaped crystals. As compared to this phloem, the interxylary phloem was characterized by having parenchyma cells larger

in diameter (Fig. 37B, C) and higher in length. The interxylary phloem was composed of sieve tube elements, companion cells, axial and ray parenchyma cells. Length and width of interxylary sieve tube elements were measured about 372-385 μm and 26-30 μm respectively, while it was 364-371 μm and 22-28 μm respectively for external phloem (Table. 7). Rays were mostly uniseriate but occasionally bi- to tri-seriate rays passing through the phloem islands may be seen in transverse view. These rays were thin walled in the phloem islands and thick walled outside the phloem islands, in the secondary xylem (Fig. 37B). Accumulation of more and more secondary phloem in the older phloem islands exerted a pressure on the earlier formed phloem islands thus leading to crush and obliterate the non-functional sieve tube elements (Fig. 43B, C).

Structure of the interxylary phloem islands: Interxylary phloem islands were arranged in an irregular way (Fig. 10A) and they were smaller and fewer centripetally while considerably larger and more numerous towards the periphery of the stems. Occasionally larger islands may be seen in the inner part of the stem (Fig. 10A). These islands were tangentially elongated and more or less oval to oblong in shape. With the advancement of secondary growth, the islands became older and embedded deep inside the thick walled xylem derivatives. Therefore, sieve tube elements became non-functional and showed heavy accumulation of callose. Consequently, they became crushed and underwent obliteration (Fig. 43B, C). The cambial arcs embedded to the inside margin of each phloem islands continued to divide periclinally for a fairly long period producing more phloem cells. Since islands were surrounded by thick walled lignified xylem elements, the new phloem cells had no chance to expand outwards. Thus, newly formed secondary phloem always crushed the older one, especially the sieve tubes and companion cells. Accumulation of crushed and obliterated phloem cells as a cap on outer border of each island can be seen very well in the older phloem islands of thicker stems (Fig. 43C).

3.5.4 *Calycopteris* Type:

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).

3.5.4.1 *Calycopteris floribunda* Lamk. (Combretaceae)

Common Names: *Ukshi, Paper flower climber* and *Jhaal.*

Anatomy of young stem: In the young stems, the epidermis consisted of single compact layer of isodiametric, oval to oblong and thin walled parenchyma cells. Epidermis was followed by 2-3 layered hypodermis composed of parenchyma cells in which some of them were filled with tannin. The bulk of cortex consisted parenchyma cells. Several vascular bundles were joined by interfascicular cambium and formed a continuous ring of the vascular cambium. Pith was characteristically triangular in shape and showed accumulation of phenolics/tannin in the parenchyma cells but its deposition was comparatively less than the cortex.

Development of included phloem: Several collateral vascular bundles were joined together by the interfascicular cambium and formed a complete ring of vascular cambium. This ring was functionally bidirectional and produced secondary xylem centripetally and secondary phloem centrifugally. After a definite period of cambial activity (i.e. after the formation of approximately 32-35 xylem derivatives) small segments of cambium began to divide rapidly and the cambial zone became wider with 6-7 cells in each radial files (Fig. 44A). Cells in the middle of the cambial zone of relatively wider cambial segments started expanding, thus pushing the adjacent cambial cells away from each other (Fig. 464, C). Differentiation of parenchyma cells in the middle of the cambial zone consequently separated the cambial zone into outer and inner cambial zones (Fig. 44B-D). From these two segments, the inner one became functionally unidirectional and produced only phloem elements centrifugally while it did not produced any xylem elements. The outer segment however, remained functionally bidirectional and produced secondary xylem centripetally and secondary phloem centrifugally (Fig. 44E). Therefore, outer segment remained continuous with the rest of the cambium keeping the original circular outline of the cambial cylinder intact.

Cells responsible for the separation of cambial zone into outer and inner segments were differentiated into thin walled parenchyma cells. These cells were radially elongated and relatively larger in size as compared to the cells produced by adjacent cambial region (Fig. 44D-E). The first cells to differentiate from the outer segment are always radially flat cells that are transformed as such into thick walled

elements without undergoing any further changes (Fig. 44B, D). As secondary growth progressed further, lumen of these cells was completely filled by deposition of lignin (Fig. 44E). Development of secondary xylem from the outer cambial segment consequently embedded later type of segment into thick walled xylem derivatives (Fig. 45A-C). As mentioned earlier that after splitting of the cambium, inner segment became inactive. This is however, only a temporary phase that is soon reactivated and functionally become unidirectional and produced only phloem elements. Reactivation of inner unidirectional segment occurred only after the initiation of lignification in xylem derivatives produced by outer segment (Figs. 44E, 45A). Development of phloem from the inner cambial segment exerted a pressure on the thin walled cambial derivatives thus pushing them in centrifugal direction. This process (i.e. formation of outer and inner cambial segments and development of its derivatives) is repeated several times. Consequently this led to number of islands of thin walled tissue/phloem embedded in thick walled xylem derivatives.

Sieve tubes were rarely formed in the external secondary phloem (Fig. 44C). Therefore, most of the sieve tube elements were developed in the included islands. Phloem regions external to the bidirectional cambial ring were mostly composed of parenchyma cells that showed heavy accumulation of phenolic compounds making it difficult to identify the cell boundary from the adjacent ones (Fig. 44A, B, D). The included phloem was composed of sieve tube elements, companion cells, axial and ray parenchyma cells. Length and width of the sieve tube elements was about 467 to 489 μm and 23 to 28 μm respectively. There was no much difference in the length of width of external (470 to 489 μm) and included sieve tube elements (Table. 7). Uniseriate rays passing through the phloem islands were thin walled and radially elongated (Fig. 44D, E) where as they were thick walled in the secondary xylem. As more and more secondary phloem was accumulated in the phloem islands, ray cells changed their shape and became oval to oblong and elliptic (Fig. 44E). In the innermost phloem islands ray cells were completely obliterated along with the sieve tube elements (Figs. 45F, 46A).

Structure of the interxylary phloem islands: Interxylary phloem islands were arranged in an irregular fashion (Fig. 45D) and they were smaller and fewer in the inner portion (i.e. towards the pith) of wood while considerably larger and more numerous towards the periphery. Occasionally convergence of small islands led to the

development of larger islands in the inner part of the wood (Fig. 45D). These islands were usually tangentially elongated and had no definite shape. Occasionally the adjacent phloem islands were found inter-connected with each other by thin walled parenchyma cells that run parallel to thick walled xylem rays (Fig. 45E). At such places rays were mostly thin walled and bi-to triseriate and free from the tannins. As secondary growth progressed further, the islands became older and were embedded deep inside the stem. The sieve tube elements in these phloem islands became non-functional and showed heavy accumulation of callose. They became crushed and were obliterated (Figs. 45F, 46A). The segments of the cambium present on the inner margin of each island produced new cells that formed sieve tube elements and companion cells. Since, islands were surrounded by thick walled lignified elements, the expanding phloem elements were unable to spread outwards. Therefore, the pressure exerted by newly added phloem derivatives was directed centrifugally and in due course, radially elongated parenchyma cells also became crushed (Figs. 45F, 46A). These unidirectional cambial arcs continued to divide for a relatively long period of time and the newly formed secondary phloem cells crushed the older ones especially the sieve tubes and companion cells. Eventually inner cambial segments ceased to divide and the cambial initials differentiated (Fig. 46A).

Structure of the secondary xylem: The secondary xylem was diffuse porous with indistinct growth rings. Several irregularly arranged phloem islands of various shapes and dimensions were embedded in thick walled xylem derivatives (Fig. 45D). The xylem was composed of vessels (both wider and fibriform vessels), libriform fibres, axial and ray parenchyma cells. Wider vessels were mostly solitary and possessed simple perforation plate on their oblique end walls. On the other hand, fibriform vessels were imperforate tracheary elements except for the occurrence of a small sub-terminal perforation plate near each end of the cell. Therefore, it was difficult to distinguish them in transverse view. Considerable variation was observed in the length and width of vessel elements and fibriform vessel elements length and width.

Even after the deposition of secondary wall material (especially lignin), libriform fibres retained their living protoplast and nucleus not only in the outermost wood but also in the innermost part of 4-5 year old xylem (Fig. 46B, C). These fibres were characterised by presence of thick lignified walls with narrow lumen and tapering ends. The pits on the radial walls were simple and the pit apertures formed a

narrow angle with the fibre axis. Shape of the nuclei differed from oval to circular or elliptic and oblong measuring about 5.6-7.3 μm in length and 1.8-5.6 μm in width. Accumulation of starch in the fibre lumen was common in all the samples investigated (Fig. 46B). Fibres in the current year's growth were usually free from the starch grains while heavy accumulation of starch was observed in the inner growth rings. Xylem fibres were 2-2.5 times longer than the fusiform cambial cells (Table. 7) and measured between 883 to 927 μm and 18 to 22 μm in length and width respectively.

Xylem rays were uniseriate and heterocellular, in which terminal ray cells were procumbent and elongated while rest of them were more or less isodiametric. They had thick or thin walls with relatively large, oval to circular simple pits. Some of the ray cells were also differentiated into perforated rays (Fig. 46D-F). They were mostly oval to circular or elliptical when formed in the middle of rays while those differentiating from the terminal ray cells were vertically elongated and longer than the isodiametric ones (Fig. 46E-F). As compared to adjacent ray cells, perforated ray cells were usually larger in size but several times shorter, smaller than the vertically arranged vessels. They were more or less isodiametric and possessed simple perforation plates. The perforations were oval, circular or elliptical. Although, they were developed from terminal or central ray cells, they were always observed in contact with axial vessel elements.

3.6 CAMBIUM NORMAL IN FUNCTION, ABNORMAL IN CONFORMATION

Sometimes, outline macro-morphology of the stem differs from the normal circular condition therefore; in transverse view the stem becomes different in its conformation (i.e. different in shape in transverse view). In such cases the vascular cambium becomes functionally normal producing secondary xylem centripetally and secondary phloem centrifugally but show unequal cambial activity. Due to unequal activity of the cambium at certain segments, it results into macro-morphological abnormalities in the stem structure. The cambium has a three dimensional shape other than cylindrical, and may be either single or multiple cambia, but in which successive cambia are not involved. The multiplicity of cambia in this category arises either from simultaneous

origin in several sites or from fracture of the cambium into several portions (Carlquist 1988). This category may be further divided into following subtypes:

3.6.1 Stem Flattened In Transaction:

Occurrences of flattened stems in the stems of several climbers have been documented in various text books and research manuscripts (Schenck 1893; Solerder 1908; Eames and McDaniels 1947; Carlquist 1988, 2001, 2007; Isnard and Silk 2009). In this form, a single vascular cambium divides unequally resulting into flattening of the stem by deposition of more xylem on one or two sites of the stem. Thus, the stem becomes flattened unilaterally if only one site receives a greater deposition of secondary xylem, or bilaterally, if two sites receives a greater deposition. Although flattened stem occurs in free-standing situations, the flattened stem seems related to the climbing habit of lianas in that the broad side of the flattened axis rests on branches of trees that support a liana (Carlquist 1988).

3.6.1.1 *Camptosema isopetalum* (Lam.) Taub and *Phanera glabra* Jacq.

***Camptosema isopetalum* (Lam.) Taub** (Fabaceae) and
***Phanera glabra* Jacq.** (Leguminosae)

Morphology of the stem: *Camptosema isopetalum* and *Phanera glabra* (Leguminosae) are perennial climbers which may be found spreading on the ground in absence of a supporting object/host. In the young plants, stem was circular in outline, but when the stem exceeds 1.5 to 2 mm in diameter, it gradually became flat (Fig. 47A, B, 48A-C). In both the species, flattening of the stems was achieved by the deposition of more secondary xylem by the single vascular cambium on two opposite lateral sides of the climbing plants (Fig. 47B, 48C, E-H).

Structure of cambium: The cambium was storied in *Camptosema* and semi-storied in *Phanera* with relatively short fusiform cambial cells varying from 198-212μm and 227- 249 μm in length and from 17-21 μm and 24-29 μm in width in *Camptosema* and *Phanera* respectively. In transverse view, the resting cambium of both the species appeared two- to three-layered and four- to six-layered when active. Cambial rays were uni- to biseriate and heterocellular while multicellular rays were observed occasionally.

Anatomy of stem: A single cell-layered epidermis, composed of tangentially arranged barrel-shaped cells, enclosed 2-3 layered hypodermis beneath it. A multilayered cortex, composed of thin-walled parenchyma cells, was delimited by a continuous layer of perivascular fibers, while endodermis and pericycle were indistinct. Several collateral vascular bundles were joined by interfascicular cambium and formed the first cylinder of vascular cambium, which was functionally normal, but also bidirectional, producing secondary xylem centripetally and secondary phloem centrifugally. Uniform activity of the cambium maintained the circular outline of the stem until it became 1.5 to 2 mm in diameter. However, flattening of the stem was controlled by the pattern of cell division in the cambial zone. Shift from spreading to climbing habit resulted in loss of circular outline to ribbon like flat stems due to unequal activity of the vascular cambium (Fig. 47B, 48B,C). The portions of the cambium facing to contact side and its opposite side of the supporting object (referred as proximal end) became unidirectional and do not deposit secondary xylem once the plant adapt to climbing habit (Fig. 48C). On the contrary, cambium portion located away from the contact side (referred as distal part) of the stem was relatively more active thus the stem ultimately became flattened like ribbon. At the proximal sides, xylem and phloem rays run relatively parallel to each other from the pith to periphery. The portion between proximal and distal sides of the stem showed increase in tangential distance between the adjacent rays (Fig. 47C-E). Not only the distance between adjacent rays increased but additional rays were also developed in between the rays. Similarly, number of xylem derivatives in the radial file also increased from the distal to proximal end of the stem.

Structurally, secondary xylem was composed of vessel elements (both wider and fibriform), fibres, axial and ray parenchyma cells. Unlignified axial parenchyma formed continuous tangential bands. Whereas, secondary phloem was composed of sieve tube elements, companion cells, and axial and ray parenchyma cells.

3.6.2 Stem Lobed In Transection:

3.6.2.1 Stem Lobed But Not Fragmented:

If a single cambium deposits secondary xylem in greater quantities at three or more sites thus, the stem becomes lobbed as it receives a greater deposition of secondary xylem and the stem becomes lobbed configuration. Such lobbed configuration of the

stem is not confined only to the lianas but they characteristically occur in buttressed trees of rain forest areas (Francis 1924).

3.6.2.1.1 *Phanera outimouta* (Aubl.) Queiroz (Leguminosae)

Morphology of the stem: *Phanera outimouta* is a perennial climber in which the young stem was circular in outline, but when the stem exceeds 3 to 5 mm in diameter, it gradually became square. With the advancement of secondary growth only certain segments are functionally active while rest of the segments of it became unidirectional. Therefore, thickening of the main stems was achieved by the deposition of more secondary xylem by the single ring of vascular cambium only on four sides of the stem, consequently resulting into lobbed stem.

Structure of cambium: The cambium was semi-storied with relatively short fusiform cambial cells varying from 218-262µm in length and 24-29 µm in width. In transverse view, the resting cambium was two- to three-layered and four- to six-layered when active. Cambial rays were uni- to biseriate and heterocellular while multi-cellular rays were observed occasionally.

Anatomy of the stem: The young stem of *Phanera outimouta* was made up of single layer of thin walled more or less barrel shaped epidermal cells. Epidermis was followed by hypodermis consisting of 2-3 cell layered chlorenchyma cells while cortex was composed of thin walled oval to polygonal parenchyma cells. Differentiation of pericyclic fibre bands was observed only after the fifth internode. At this stage, accumulation of tannins began in the cortical parenchyma and pith cells. Several conjoint, collateral vascular bundles were joined by interfascicular cambium and formed the complete ring of cambium. This cambium was functionally bidirectional producing xylem centripetally and phloem centrifugally. Stem maintained its radial arrangement up to 3 to 5 mm in diameter. Thereafter, cambial cell division became unequal producing more secondary xylem on four corners while rest of the cambial segments was functionally either sluggish or ceased to divide (Fig. 48D).

Unlike other species (*Phanera glabra*), irrespective to the position of the stem in relation to supporting object, in *P. outimouta* it increase in thickness only at four side of the stem. Formation of the lobbed stem was entirely controlled by the pattern

of cell division in the cambial zone (Fig. 48D). The young stem were initially oval to circular in outline due to the normal activity of the cambium but very soon cell division activity remained confined to small portions of cambium located to opposite to each other. Rest of the segments of the cambial either became sluggish or very slow; therefore, deposition of secondary xylem and phloem took place only at four side of the stem, which ultimately results into cruciform shape of the stem.

3.6.3 Stems with Furrowed xylem:

In the members of Bignoniaceae, small segments of cambium become functionally unidirectional after production of certain amount of secondary xylem and phloem. After the production of 0.9-1.5 mm of secondary xylem, small segments of the cambium ceased to divide centripetally. Due to cessation of secondary xylem production, unidirectional segments of cambium lag behind while production of only sieve tube elements continued (Carlquist 1988, 2001a; Lima *et al.* 2010; Pace *et al.* 2011). Chalk and Chattaway (1937) have termed this phenomenon as "Interrupted stem". Species with furrowed xylem show conversion of cambium to unilateral (producing phloem only) or nearly unilateral (only a little secondary xylem compared to the abundant secondary phloem production at particular sites). In the *Phaedranthus* the four sites where furrows were formed represent early conversion of cambium to unilateral activity, and no further sites for unilateral cambial activity are developed, so that the xylem cylinder is normal except for the deep grooves at those four points (Carlquist 2001a, Fischer *et al.* 2004). In other members such as *Dolichandra anguis-cati*, *Adenocalymma divaricatum*, *Pithoctenium* and *Lundia* etc., of the Bignoniaceae, show additional sites convert to unilateral cambium activity in between the original sites, so that shallower grooves in addition to the deeper ones are present (Pfeiffer 1926). Another variant on the grooved xylem habit is shown by *Passiflora glandulosa* (Ayensu and Stern 1964). In these plants, conversion of cambium to unilateral (or nearly unilateral) activity occurs progressively; it begins at five sites, then additional segments of cambium are converted on the margins of the grooves, so that the grooves become wider and wider. In the present investigation, *Bignonia alliacea* L. is studied as an example of furrowed xylem, which showed four phloem wedges.

3.6.3.1 *Begnonia alliacea* L. (Bignonieae)

Common Names: *Garlic vine, Lataparul* and *Chanamlei.*

Anatomy of young stem: Young stem of *Bignonia alliacea* was oval to circular in outline. Anatomy of the young stem revealed that a single layer epidermis covered with unicellular trichomes was composed of oval to polygonal compactly arranged parechymatous cells. Beneath the epidermis lie a 2-3 layers of thin parenchymatous hypodermal cells. Hypodermis was followed by several cell layer thick parenchymatous cortex. Pericyclic fibres formed a continuous layer or cap outside the primary phloem (Fig. 49A). Six to seven conjoint, collateral vascular bundles were joined by interfascicular cambium and formed a complete ring of the vascular cambium. From these vascular bundles, four of them were well developed and deposited greater amount of xylem and phloem (Fig. 49A, B). Formation of phloem wedges initiated from the sixth node of young stem (Fig. 49B).

Structure and development of Phloem wedges: The vascular cambium was functionally normal in action throughout the cambial circumference producing secondary xylem centripetally and phloem centrifugally. Formation of phloem wedges started from sixth visible internode when there was about 2-3 mm of xylem was accumulated. The cambial ring that was appearing cylindrical configuration at first gradually lost its circular outline subsequently with the initiation of grooves formation (Fig. 49A-C). Development of phloem wedges resulted due cessation of cell division activity of cambium towards centripetally. Small segments of the cambium opposite to the four major vascular strands became functionally unidirectional producing only secondary phloem with no cell division and differentiation towards the inner side (Fig. 49C). On the contrary rest of the cambial segments remained functionally bidirectional therefore, the portion of the cambium that ceased to divide towards inner side lag behind while other segments were progressively pushed forward due to the addition of secondary xylem (Fig. 49C). Such behavior of the cambium i.e. unidirectional segments of cambium alternating with functionally bidirectional cambium ultimately resulted into four grooves (Fig. 49E, F).

As the secondary growth progressed further, additional four wedges of phloem were formed from the bidirectional cambium. With the increase in stem diameter formation of such phloem wedges continued which ultimately resulted into four furrowed xylem (Fig. 49D). Rays embedded in the secondary xylem were mostly uniseriate but at lateral side of phloem wedges they were multiseriate (Fig.49E, F).

Interestingly, in each furrow their edges run parallel and maintained uniformity in their width.

Structurally the secondary phloem produced in the wedges and by functionally bidirectional cambium (normal) was composed of sieve tube elements, companion cells, phloem fibres, axial and ray parenchyma cells. However, the sieve tubes elements differentiated from the unidirectional cambium were larger (28-38μm) in diameter as compared to that of phloem (20-26 μm) produced by the cambia having normal position. In phloem wedge, bands of fibres differentiated in an alternating pattern with bands of sieve tubes elements (Fig. 49E, F). These bands of fibres were secondary in origin and not directly differentiated from the cambial initials. During this process, fibre differentiation usually progressed from the rays bordering the furrows toward the centre of the furrow (Fig. 49F), but in rare instances differentiation of these fibres was observed from the centre toward the edges. The fibres which differentiated in the phloem produced by functionally bi-directional cambial arcs were also arranged in bands alternating with sieve elements and parenchyma. Contrary to the furrowed phloem, in normal phloem these fibres differentiated simultaneously rather than in one direction or another. The phloem fibres were measured from 750-1100 μm and 12-25 μm in length and width respectively.

The secondary xylem was diffuse porous and composed of fibres, vessels, axial and ray parenchyma. Both wider vessel elements as well as very narrow fibriform vessels members were observed in the sample studies. They were mostly solitary with simple perforation plate on their slightly oblique to transverse end walls. The narrow vessels (also termed as fibriform vessels) were indistinguishable in transverse view. Fibriform vessels were like imperforate tracheary elements except for the occurrence of a small sub terminal perforation plate near each end of the cell. Their length and width was measured from 250-425 μm and 95-152 μm respectively
While fibriform vessel were 412-687 μm and 25-50 μm in length and width respectively.

3.6.4 Xylem in Plates:

In some dicotyledonous wood in which fascicular areas are few and are separated by large ray areas that consist of thin-walled parenchyma constitute a kind of anomaly

with respect to cambial activity (Pfeiffer 1926; Metcalfe and Chalk 1950). The occurrence of non-lignified xylem is cited by Metcalf and Chalk (1950) as an anomaly in some species of *Breweria, Convolvulus, Exogonium,* and *Ipomoea* (Convolvulaceae). Carlquist (1988) is of the opinion that such pattern of cambial activity should not be considered as a cambial anomaly, and perhaps should not be listed as an anomaly at all because cambium is functionally normal i.e. bidirectional only difference is related with the formation of lignified and non-lignified tissues. Certainly one could cite herbaceous annuals in which this occurs. Xylem in plates is studied in *Aristolochia indica* Linn., and *Tinospora cordifolia* (Thunb) Miers.

3.6.4.1 *Aristolochia indica* Linn. (Aristolochiaceae)

Common Names: *Indian birthwort, Garalika, Garudi and Isvarmul.*

Anatomy of young stem: In *Aristolochia,* epidermis of the young stem was made up of single layered thin walled parenchyma cells and covered with thick cuticle and unicellular trichomes. The epidermis was followed by 2-3 layers of hypodermis. Cortex was composed of parenchyma cells. In the young stem, eight conjoint collateral open vascular bundles were joined by the interfascicular cambium and formed the complete cylinder of it (Fig. 50A). On the outer side (towards epidermis) each vascular bundle was separated from the cortex by bundle caps (i.e. perivascular fibres) fibres while they were separated from each other by large multicellular medullary rays (Fig. 50A).

Structure and development of xylem plates: In *Aristolochia indica* secondary growth in the main stem was achieved by the normal activity of the vascular cambium that formed secondary xylem centripetally and secondary phloem centrifugally. During the course of cell division and differentiation, fascicular segment of the cambium produced thick walled lignified elements while interfascicular segments of the cambium exclusively differentiated into thin walled unlignified parenchyma on both inner and outer side of the stem (Fig. 50C). As the stem increased in diameter, circumference of the cambium was also increased but as the lignified elements differentiation was restricted to smaller segments of the cambium, thick walled xylem appeared as plates embedded in thin walled parenchymatous background (Fig. 50B-D). With the advancement of secondary growth, additional rays were also developed in the segment of cambium that was producing thick walled lignified xylem elements.

These newly developed rays were uni- to biseriate in the initial stage but they also became multiseriate with further increase in stem diameter. Similar to medullary rays, these newly developed rays also formed thin walled ray parenchyma on both external and internal side of the cambium (Fig. 50C). Such pattern of cambial derivative differentiation resulted into embedding of thick walled lignified xylem plates into a bulk of thin walled parenchymatous background. This phenomenon occurs frequently in the xylem which ultimately results in to plates in xylem (Fig. 50B, E).

The xylem plates were composed of vessels, fibres, narrow sheath of vascicentric axial parenchyma and some apotracheal parenchyma cells. In transverse view these xylem bands were frequently interrupted by large thin walled rays which often developed as into polycentric rays i.e. ray cells dedifferentiate into meristematic centers at several places (Fig. 50B-D). In tangential view they were heterocellular compound and tall. The ray cells were procumbent, though square and upright cells were especially found on the ray periphery. Ray cells were 22.8 to 29.6 µm diameter and no uniseriate rays or uniseriate wings were observed on multiseriate rays. Very wide multiseriate rays were 850-1230 µm in height and 18-50 cells wide.

Vessels were mostly solitary but radial or tangential multiples of 3-5 vessels were also observed occasionally. Usually vessels were oval to oblong in cross section. Vessel elements showed presence of dimorphism i.e. wider and fibriform vessels. The wider vessels were 337 to 687 µm in length and 175 to 312 µm in width and possessed simple perforation plate on slightly oblique to transverse end walls. Fibriform vessels were 500 to 712 µm in length and 21-31 µm in width and possessed obliquely placed simple perforation plates on their end walls. The bordered pits on the lateral walls were alternate with oval to round in shape, sometimes approaching rhomboidal with narrowly oval aperture of 5 to 7 µm in diameter.

3.6.4.2 *Tinospora cordifolia* (Thunb.) Miers (Menispermaceae)

Common Names: *Heartleaf moonseed, Guduchi, Giloya and Gudvel.*

Anatomy of young stem: In the young stems, thin cuticle covered the epidermis, which was made up of consisted of single compact layer of isodiametric and thin walled parenchyma cells. A 2-3 cell layered hypodermis composed of parenchyma cells differentiated beneath it while the cortex was composed of bulk of thin walled

parenchyma cells. Cortical cells showed heavy accumulation of oval to circular starch grains. Five to six vascular bundles were joined by interfascicular cambium and formed a ring of the cambium. Cambial rays were large, heterocellular and compound with procumbent cells. Pith was relatively small and consisted of parenchymatous cells showing heavy deposition of starch grains.

Structure secondary xylem and xylem plates: Secondary growth in the main stem was achieved by the single ring of vascular cambium which remained functional throughout the lifespan of the plant. The cambium was functionally normal and formed secondary xylem centripetally and secondary phloem centrifugally. Secondary xylem was with large vessels and wider rays (Fig. 50E, F). In transverse section, axial elements of secondary xylem plates were frequently separated by large rays. These rays often developed meristematic centers (referred as polycentric rays) i.e. small group of ray cells de-differentiate into meristematic cells at several places (Fig. 50F). Rays were measured about 1450 to 2300 μm in height and 300-390 μm wide. In tangential view they were heterocellular and most of the rays were procumbent, while square and upright cells were especially found on the ray periphery. Ray cells were 32.2to49.5μm diameter while no uniseriate rays were observed.

Fibre tracheids were 600-700 μm in length and 25-30 μm in width. Vessels were mostly solitary but radial or tangential multiples of 3-5 vessels were also observed infrequently. Vessels were mostly oval to along in cross section, and often obstructed by numerous tyloses, which some time completely blocked the lumen. Such tyloses often showed deposition of prominent starch granules. On the basis of their diameter, vessels may be categorised into wider and narrower vessel elements. Length of the wider vessel elements was less than its diameter. They were measured from 165to300 μm in length and 170 to 425 μm in diameter while narrower vessel elements were longer than their diameter i.e. they were 237-300 μm in length and 62-100 μm in width. Vessels possessed simple perforation plate on their slightly oblique to transverse end walls alternate bordered pits. They were oval to oblong, sometimes approaching rhomboidal with narrowly oval aperture of 6 to8 μm wide and 7 to 10 μm in vertical diameter.

3.6.5 Divided Xylem Cylinder:

This term is applied to those species in which the stem has a vascular cylinder that is furrowed. During secondary growth, each of the furrows became surrounded by cambium and in subsequent stages of secondary growth; additional secondary tissues are deposited to each of the xylem segments independently. This cambial variant is also known as "cleft xylem mass" and it was originally studied by Radlkofer (1875) in *Urvillea* (Sapindaceae). This cambial variant is also known in other species of the Sapindaceae. In the present study, development of divided xylem is studied in *Serjania corrugata.*

3.6.5.1 *Serjania corrugata* Radlk (Sapindaceae)

Anatomy of young stem: in the young stems, the shoot apex was circular in outline but it gradually became lobbed and distinct five lobes may be observed from the second visible inter node onwards. These lobes were delimited by well developed furrows but as secondary growth progress further they were gradually attenuated so that the mature stem became slightly lobbed in outline. A single layer of oval to polygonal cells composed the epidermis while the cortex was made up of 3-4 cell layered at furrows and 8-12 at the lobes. Some of the cortical parenchyma differentiated into secretary cells. Accumulation of phenolics compounds was also observed in some of the cortical cells. As compared with cortical parenchyma, an endodermis was made up of relatively larger cells. Beneath the endodermis, a single layered pericycle was observed which was delimited by endodermis externally and protophloem internally.

Establishment of vascular cambium and differentiation of vascular elements: In the 4th inter node, procambial strands began to appear and they were prominent in the 5^{th} inter node. Each lobe has its own vascular arch with 2-3 central vascular bundles and two lateral vascular bundles. In between these vascular bundles, interfascicular cambium began to appear and between 7^{th} and 8^{th} internode all the vascular bundles were joined by interfascicular cambium and formed a complete ring of vascular cambium in 10^{th} inter node. However, differentiation of vascular elements was not equal throughout the cambial arc. Initially differentiation of thick walled elements was restricted to central vascular bundles while newly formed interfascicular segments of vascular cambium produced only phloem elements. Differentiation of

vascular elements from the lateral vascular bundles was relatively slow while it was more and faster in central vascular bundles. In the region of the furrows, the cambium is unidirectional, producing only externally a small amount of phloem. Parenchyma cells of pericyclic in origin fill up the region between the small xylem masses. Periclinal divisions of these cells expand this tissue radially, so in later stages the furrows become less distinct. Initially, more parenchyma cells than vessel elements and fibers are produced. The vessel elements initially formed have smaller diameters than those formed later.

The cambium almost completely circles the xylem of the lobes, except in the regions that connect the core of the lobes with the pith (Fig. 51B, F). These connections may persist in old stems. As a consequence of the production of vascular tissues, the cells of these connections show intense cellular division, in order to remain connected with the pith of the xylem masses. At this stage, in each lobe the xylem mass was almost completely surrounded by its own cambium. In certain regions of each cambium, development of large parenchymatous rays was also observed.

Structure of secondary xylem: The secondary xylem was diffuse porous with indistinct growth rings. The xylem was composed of vessels (both wider and fibriform), libriform fibres, axial and ray parenchyma cells. Rays were mostly uni-biseriate while multiseriate rays were observed occasionally. Vessels were mostly solitary and possessed simple perforation plate on their oblique end walls. It was difficult to distinguish the fibriform vessels in transverse view due to their smaller diameter. Considerable variation was observed in the length and width of vessel elements and fibriform vessel elements length and width.Vessels may be categoried into two types (fibriform and wider) on the basis of their diameter. The wider vessels were measured about 263 - 387 μm in length and 230 - 278 μm in diameter while fibriform vessels were 339 - 387 μm in length and 15 - 35 μm in diameter. Length of fibres was ranged between 1569 - 1793 μm.

3.6.6 Compound Secondary Xylem:

In this type of unusual cambial variant, independent rings of cambia develop in cortex of a normal cylinder giving an appearance as several stems are joined together. The three dimensional course of vascular bundles has been studied in *Serjania caracasana*

of the Sapindaceae (Carlquist 1988).In the stems of the *Serjania caracasana* this cambial variants represented by a central vascular cylinder surrounded by eight peripheral vascular cylinders.

3.6.6.1 *Serjania caracasana* (Jacq) Willd (Sapindaceae)

Anatomy of young stem: In the apical shoot and young stems of *Serjania caracasana*, a thin walled epidermis made up of barrel shaped cells enclosed 2-3 cell layered parenchymatous hypodermis. Just beneath the hypodermis, a several cell layered cortex was differentiated. In the 3rd visible node, protophloem and protoxylem began to differentiate. As compared to cortical parenchyma, an endodermis was made up of relatively larger cells. On the inner side of the endodermis, a single layered pericycle consisting of thin walled cells was observed. The pericycle was delimited by the endodermis externally and protophloem internally. The young stem was composed of central cylinder of cambium which formed the main stem while outside to it, in the cortical region eight more cylinders were developed which encompassed the central cylinder. This arrangement of vasculature gives an impression that nine stems are merged together to form one complete stem.

Origin of vascular cambium: In the 3rd visible internode protophloem and protoxylem began to differentiate. In the next internode, a procambial strands began to appear in between the protoxylem and protophloem. Simultaneously, in the cortical region eight similar strands of vascular bundles began to differentiate. Although, all nine vascular cylinders (eight on the periphery and one in the centre) began to differentiate concomitantly, the central one is larger in size because of larger pith. In the 4th and 5th internode a prominent procambium was visible with protoxylem and protophloem elements on either side. In the 7th inter node the parenchyma cells between the adjacent vascular bundles acquired the meristematic activity to connect the fascicular regions of the cambium. A well established vascular cambium may be seen in the 10th inter node in all the nine vascular cylinders. The vascular cambia of all the cylinders were functionally bidirectional and produce secondary xylem centripetally and secondary phloem centrifugally.

Structure of xylem: Secondary xylem of all the vascular cylinder was diffuse porous with indistinct growth rings. It was composed of vessels, fibres, and axial and ray parenchyma cells. Rays were mostly uni-biseriate while multiseriate rays were

observed occasionally. Vessels may be categorized into two types (fibriform and wider) on the basis of their diameter. The wider vessels were measured about 248 - 394 μm in length and 190 - 285 μm in diameter while fibriform vessels were 350 - 400 μm in length and 15 - 35 μm in diameter. Vessels were mostly solitary but radial multiples of 2-3 vessels were also observed while it was difficult to distinguish the fibriform vessels in transverse view due to their smaller diameter. All the vessels possessed simple perforation plate on their slightly transverse to oblique end walls. Length of libriform fibres was ranged between 1539-1843 μm.

3.7 COMBINATIONS OF CAMBIAL VARIANT

During the present study, some of the samples showed combination of more than two different types of cambial variants. In such cases it is difficult to place them in any of the single type, thus they are grouped in to the category named "combination of cambial variants". For example, flattened stems were 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 eccentric rings of successive cambia. This combination is illustrated by Pfeiffer (1926) for *Machaerium* of the Fabaceae, *Anomospermum* (Menispermiaceae) and *Securidaca* (Polygalaceae). In the present study, *Macherium aculeatum* (Fabaceae), and *Securidaca rivinaefolia*, of the Polygalaceae (collected from Cerrado Forest, Sao Paulo State of Brazil) showed flattened stem with eccentric successive cambia. *Ipomoea hederifolia* and *I. quamoclit* showed development of successive cambia along with the presence of inverse cambia/vascular bundles and interxylary (included) phloem development.

Plants (such as *Ipomoea hederifolia, I. quamoclit, I. triloba, Cocculus hirsutus* and *Coccinia indica*) included in this part are explained elsewhere, thus they are described in brief while *Macherium aculeatum* (Fabaceae) and *Securidaca rivinaefolia* (Polygalaceae) are described in detail.

3.7.1 *Cocculus hirsutus* (L.) Diels. (Menispermaceae)

[Successive cambia + Flattened stem]

Cocculus hirsutus, shows flattened stem with successive cambia. In the early stages of stem development several collateral vascular bundles are joined by inter-fascicular cambium resulting in the formation of a complete cylinder (Fig. 20A, 21A). After

functioning for two to three years, the cambial ring was ceased to divide. Subsequently a second ring of cambium was formed from the pericyclic parenchyma cells (Fig. 21B-D). These parenchyma cells underwent periclinal divisions and gave rise to cells that became lignified, abaxially, and cambial cells adaxially. The cambial cells divided periclinally formed to individual vascular bundles with xylem and phloem. Later the cambium in each bundle was joined by interfascicular cambium. Subsequent cambia developed similarly resulting in the formation of successive rings of xylem and phloem (Fig. 20A-C, 21B). As secondary growth started further where stem have taken support that portion results in to flattened stem (Fig. 20D). Here in case of *Cocculus* stem shows eccentric successive cambia with flattened stem (Fig. 20D).

3.7.2 *Coccinia indica* L.(Cucurbitaceae)

[Intraxylary phloem + Lobed stem]

Stem anatomy and the development of intraxylary phloem were investigated in six to eight years old *Coccinia indica* (Cucurbitaceae). Thick stems of *Coccinia* showed lobed stem but it was fragmented (Fig. 38A). Secondary growth in the stems was achieved by the normal cambial activity. In the innermost part of the thicker stems, xylem parenchyma and pith cells dedifferentiated into meristematic cell at several points. In some of the wider rays, ray cells de-differentiate and produced secondary xylem and phloem with different orientations and sometime a complete bicollateral vascular bundle. Inner cambial segment of the bicollateral vascular bundle (of primary growth) maintained radial arrangement even in the mature stems but at most of the places they were functionally either inactive or very slow. Concomitant to the obliteration and collapse of inner phloem (of bicollateral vascular bundles), parenchyma cells encircling it became meristematic forming a sheath of cambia. In the later stage of secondary growth, the internal cambium was originated that produce intraxylary secondary phloem centripetally. Secondary xylem consisted mainly of axial parenchyma, small strands of thick walled xylem derivatives embedded in parenchymatous ground mass, wide and tall rays along with exceptionally wider vessels characteristic to lianas. In thick stems, the axial parenchyma and xylem ray cells de-differentiated into meristem, which later on re-differentiated into interxylary

phloem. Present investigation also reports fibre dimorphism and presence of vestured pits in the vessels.

3.7.3 *Ipomoea triloba* L. (Convolvulaceae)

[Successive cambia + Internal phloem + Internal cambia]

Stem anatomy of *Ipomoea triiloba* showed combinations of cambial variants like successive cambia, internal phloem and internal cambia (Fig. 52A-D). Development of successive cambia was similar to the *I. hedrifolia* and *I. quamoclit*. The cambium functions in a normal way like other dicots for a short time and produces a few cell layers of secondary phloem centrifugally and secondary xylem centripetally. The secondary phloem consists mainly of sieve tubes, companion cells and phloem parenchyma cells while secondary xylem consists of vessels, fibriform vessel members, fibre tracheids, tracheids and xylem ray parenchyma cells. This cambium remained functional for definite period producing 5-6 mm of secondary xylem, which later ceased to divide. Up to this stage the protoxylem remains unchanged while clusters of internal phloem increased slightly in size due to the differentiation of additional pith cells into sieve tube elements. A second ring of cambium was developed from the axial parenchyma cells at a distance of about three to six cell layers outside the phloem produced by the previous cambium.

During the development of new cambium, one or two parenchyma layers underwent repeated divisions and resulted into formation of five to six layered wide bands of meristematic cells. This newly developed cambium was functionally normal producing secondary xylem towards the centre and secondary phloem towards periphery. In the innermost successive rings, earlier formed sieve tube became non-functional by heavy accumulation of callose followed by their obliteration. These non-functional sieve tube members were replaced by addition of new phloem elements.

In 15-20 mm thick samples, small arcs/segments of internal cambium were formed on the outer side of the internal phloem strands. This internal cambium was originated from the fully matured pith parenchyma cells by repeated tangential divisions (Fig. 52D).

3.7.4 *Macherium aculeatum* Raddi. (Fabaceae)

[Flattened stem + Successive Cambia]

Morphology of the stem: *Macherium aculeatum* Raddi. (Fabaceae) is a perennial climber which may be found spreading on the ground in the absence of a supporting object/host. When the plant is young, it is circular in outline, but when the stem exceeds 1.5 to 2 mm in diameter, it gradually becomes flat (Fig. 53A-C). Flattening of the stems is achieved by the formation of asymmetrical half-moon shaped successive cambia, but only on the lateral sides of the stem (distal ends). The portions of the stem directly in contact with the supporting object (proximal end) do not participate in the development of successive rings of the cambium. Moreover, cell divisions and differentiation of secondary xylem in this region also become very slow, thus giving the impression that the cambium is nonfunctional on the proximal ends. However, on careful observation, one can find differentiating xylem and phloem derivatives on the proximal side of the stem.

Structure of cambium: The mature stem was composed of three to five successive rings of cambia (Fig. 53C). The cambium was semi-storied with relatively short fusiform cambial cells varying from 198-212 μm in length and from 17-21 μm in width. In transverse view, the resting cambium appeared two- to three-layered and four to six layered when active. Cambial rays were uni-biseriate and heterocellular.

Anatomy of stem: A single cell layered epidermis, composed of tangentially arranged barrel-shaped cells, enclosed a 2 to 3 layered hypodermis beneath it. A multilayered cortex, composed of thin-walled parenchyma cells, was delimited by a continuous layer of perivascular fibers, while endodermis and pericycle were indistinct. Several collateral vascular bundles were joined by interfascicular cambium and formed the first cylinder of vascular cambium, which was functionally normal, but also bidirectional, producing secondary xylem centripetally and secondary phloem centrifugally. Uniform activity of the cambium maintained the circular outline of the stem until it became 1.5 to 2 mm in diameter. Thereafter, formation of secondary xylem became slow on the proximal end. On the distal sides of the stem, differentiation of xylem was increased, causing the stem to become oval to oblong and, ultimately, flat. Formation of half-moon shaped cambia on the distal ends of the stem caused additional flattening. Secondary xylem was composed of vessel elements

(both wide and fibriform vessels), fibers, as well as axial and ray parenchyma cells, while secondary phloem was composed of sieve tube elements, companion cells, and axial and ray parenchyma cells.

Structure of secondary xylem: Secondary xylem formed by all the successive segments of the cambium was diffuse porous and composed of vessel elements (both wide as well as fibriform, vessels), fibers, and axial and ray parenchyma cells. All these xylem derivatives were arranged in storied fashion. Fibriform vessels were mostly arranged in radial multiples of 3-15 cells, or formed clusters of several vessel elements, and measured 178-318 μm in length and 28-34 μm in width. Wider vessels were mostly solitary, while radial or tangential multiples of 2-3 cells were observed very rarely. Since the xylem derivatives were produced from the storied cambium, not much difference was observed in the length of either wide or fibriform vessel elements. They possessed a simple perforation plate on slightly oblique to transverse end walls, with alternate bordered pits on the lateral walls. The length of the vessel elements measured about 97 to 374 (205) μm, while their diameter measured from 101 to 284 (168) μm and 75 to 100 (84) μm for wide and narrow vessel elements, respectively. Axial parenchyma cells were septate, banded by more than 3 cells in width and diffuse-in-aggregates. Xylem fibers were non-septate, measuring from 304 to 1948 (1171) μm in length and possessing simple pits forming a narrow angle with the main axis.

Origin of successive cambia: In the apical shoot, several vascular segments joined to form the first ring of the vascular cambium that was functionally normal and circular in outline. Bidirectional and uniform cell division in the cambial cylinder of the young stem was noticed until the stem became 2 to 3 mm in diameter. By this time, plants had also started climbing on the adjacent host for support, followed by a change in pattern of cell division in the first cambium. Divisions in the cambial cells adjacent to the supporting object (proximal end/contact side) and its opposite side slowed, causing the stem to become slightly oval to oblong and, ultimately, rectangular in shape (Fig. 53A-C). As the stem became oval to oblong, some of the cortical parenchyma cells at right angle to the contact side (i.e., distal end) of the stem underwent de-differentiation on both the distal ends of the stem and formed a small segment of meristematic cells arranged in radial files like the vascular cambium (Fig. 53F). Tangentially, a very small portion of these parenchyma cells dedifferentiated

into a 6- to 8-layered band of radially arranged meristematic cells. The cells located at the midpoint of the band gave rise to the vascular cambium (Fig. 53F). The cells on the inner side of the newly developed cambial segment differentiated into conjunctive tissue, while the cells located on outer side of the cambium differentiated into pericyclic fibers and sclereids.

Newly originated cambium segments on both distal ends of the stem were functionally bidirectional, producing xylem centripetally and phloem centrifugally. At first, the cell division activity in these segments was uniform, producing identical secondary xylem throughout the segment. Thereafter, however, activity became unequal within the cambial segment (Fig. 53G). Specifically, the central part of the cambial segment divided rapidly, compared to either one of its ends, producing more secondary xylem in the middle, again compared to either of its ends. This unequal cell division in the cambial zone and differentiation of less xylem at the ends than the central part of the cambial segment pushed the cambium towards the periphery. Prior to the origin of new cambium, this variation in cambial behavior resulted in a half-moon curvature of the vascular cambium. Formation of each successive segment of cambium only on the distal ends followed a similar developmental pattern, while the proximal part remained devoid of successive cambium.

Development of vascular elements: In the young stems, flattening of the stem is controlled by the pattern of cell division in the cambial zone. Cambial cell divisions toward the proximal end of the stem became slow, as compared to distal ends, later on becoming almost unnoticeable or nonexistent. Moreover, this part of the cambium mostly divides periclinally, while anticlinal divisions are decreased or absent. This pattern of cell division pushed the cambium towards the distal ends instead of the proximal side of the stem, resulting in a flattening of the stem and the formation of half-moon shaped cambia on the distal ends. In the main cylinder, formation of more secondary xylem and phloem took place only on the distal part of the stem, while on proximal parts of the stem cambial growth was sluggish, scarcely dividing to form the secondary xylem. The presence of functional sieve tube elements and careful observation of the proximal side of the stem showed differentiating xylem and phloem.

In each newly originated cambial segment, development of xylem precedes that of phloem. The first derivatives of the secondary xylem to differentiate from these segments were always fibers, followed by the fibriform and wide vessels. As mentioned earlier, segments of cambium showed unequal activity by forming more secondary xylem in the middle portion of the cambial segment (Fig. 53E), while its formation declined gradually towards the adjacent sides (Fig. 53D). Xylem rays were mostly tall, heterocellular and uni- to multiseriate. Multiseriate rays were relatively abundant in the central cylinder, while in distal portion, they were observed only occasionally. The ray cells may be thin-walled and unlignified with distinct nucleus or they may be thick-walled, lignified and without nucleus. This variation was also observed within the same ray which was thick and lignified on the inner part of the xylem and possessed thin-walled unlignified ray cells towards the periphery. A few ray cells were square, but most were predominantly upright, while procumbent cells were observed in the middle of the rays in radial longitudinal view.

3.7.5 *Securidaca rivinaefolia* (L.) Blake (Polygalaceae)

[Lobed stem + Successive Cambia]

Anatomy of young stem: In the young stem and branches a single layered thin walled epidermis covered by thick cuticle that encloses 2-6 layered parenchymatous elongated cells (hypodermis) contained starch (Fig. 54A, B). Lenticels are present sporadically. The thickening of the cuticle is very typical due to the deposition of multilayered lignin material (Fig. 54B). In the 8-10 mm thick stems, the cuticle not only becomes strongly thickened by deposition of lignin material but also formed multi layered (Fig. 54B). It did not form a continuous layer rather it formed discontinuous patches at certain intervals, absent in the regions of depression in the stem. Formation of multilayered cuticle was observed where the epidermal cells underwent radial divisions. These epidermal cells divided repeatedly and dedifferentiated into phellogen (Fig. 54D, E) that gave rise to phelloderm (Fig. 54E) and led to breaking of multilayered cuticle (Fig. 54D). Hypodermal cells beneath the epidermis also underwent tangential elongation and divide tangentially forming more layers of hypodermal cells (Fig. 54B, C). Below the hypodermis, several layered thick cortex mostly composed of parenchyma cells were present with the sclerenchymatous

cells and gelatinous fibres (Fig. 54C). Irregular band of sclereids in perivascular position could be seen (Fig. 54C).

Development of successive cambia: The first ring of the vascular cambium remained active for a long period producing pencil thick (8-10 mm) diameter of the stem within the growing season. With the initiation of extension growth, small segments of cambium were developed only on the opposite side of the stem that was not in contact with the supporting object. The parenchyma cells in the outer region of the phloem underwent de-differentiation into meristematic cells and formed a wide band of cells by repeated periclinal divisions (Fig. 55B). From these, cells located in middle of the band remained meristematic and gave rise to the small segments of the vascular cambium. The parenchyma cells in the inner region of the newly developed cambial zone formed the conjunctive tissue while cells external to this cambial zone served the site for the origin of future cambium (Figs. 54F, 55C, E). Inception of second ring cambium began as small segments on opposite side of the stem, which gradually spread on other sides of the stem forming half-moon shaped arcs. These half-moon shaped arcs ultimately met each other to form a complete cylinder. After the initiation of new cambial segment, the parenchyma cells located in between the conjunctive tissues were formed by new cambial segments, and outermost phloem parenchyma cells formed by the previous cambial ring differentiated into tangential bands of phloem fibres. These fibre bands were interrupted by 2-3 parenchyma cells (Fig. 55D, E).

Second cambial ring remained functional for a longer period forming 2-3 mm of secondary xylem. After the formation of 38-46 xylem derivative cells next ring of the cambium was originated from the parenchyma cells located outside to the phloem produced by the previous cambium. All the successive cambia formed similar pattern of its development as described above. However, first 2-3 cambia were able to form a complete cylinder while rest of them joined to the previous cambium and never formed a complete ring (Fig. 56A). Each new cambium initiated on the opposite side of the stem and spread tangentially on both the direction thus it became half-moon shaped (Fig. 56A). Unequal activity of all the cambial segments and formation of more and more half-moon shaped cambia resulted into flattened dumbbell shaped structure (Fig. 56A). Cell division and differentiation of secondary xylem in all the segments were more and rapid in the middle of "C" shape while it declined gradually

towards adjacent sides of the segments. Such unequal activity of the cambium resulted into dumbbell shaped structure as seen in transverse view of mature stems (Fig. 56A).

Development and structure of vascular elements: The parenchyma cells between the newly developed cambium, and the phloem formed by the previous cambium differentiated into conjunctive tissue (Fig. 56B). Contribution of the newly developed cambium in formation of conjunctive tissue is very little. However, development of xylem and phloem derivatives occurred on either side of the cambium. The first elements to differentiate on the inner side of each new cambium were always fibres (Fig. 56B). Differentiation of vessels occurred only after the formation of few xylem fibres (Fig. 55D, E). However, formation of vessel elements from the newly developed cambium was also noticed occasionally (Fig. 56C).

Growth rings were distinct in the secondary xylem and were delimited by the thick walled and radially flattened latewood fibres (Fig. 57A-B). At the growth ring boundary, rays also showed distinct variation in their radial length, the ray cells were relatively elongated in the early wood, and on the other hand, at the growth ring boundary, they were more or less isodiametric forming ray nodding (Fig. 57C). As the stem showed eccentric successive cambial rings, the growth rings ran parallel with the successive rings thus they became wider in the centre and gradually became narrow towards the end.

Xylem was diffuse porous, however vessels in early wood were wider than the vessels formed in the latewood. There were a tendency towards the vessel dimorphism i.e. some of the vessels are very narrow measuring about 48-69 μm wide and 589.6 to 729.6 μm long including the tail, while length and width of the wider vessels elements is measured about 307.5 to 624.8 μm and 121.4 to 375.5 μm, respectively. The narrow vessels were longer than the wider vessel elements, and possessed a long tail on both the ends. Perforation plates were simple and arranged on the oblique end walls whereas in wider vessel elements, the tail was shorter and suddenly tapered. Like narrow vessel elements, the perforation plates in wider vessel elements were simple but more or less arranged transversely on the end walls with very short suddenly tapering tail. No fibriform vessels were observed in any of the samples studied. Vessels were mostly solitary with occasionally presence of radial or

diagonal multiples of 2-3. The non-septate fibres with distinctly bordered pits measured from 1018.31 to 1274 μm in length, and from 23.83 to 38.08 μm in width. The bordered pits measured about 4.8 to 8.2 μm in diameter (Fig. 57E).

Axial parenchyma cells were predominantly paratracheal, scanty and limited to a few cells around the vessels. Three-four cells per parenchyma strand. Rays mostly uni-seriate while biseriate rays and locally biseriate were observed rarely. Rays were about 2-16 cells in height. However, rays less than four cells in height possessed all the cells vertically upright, while isodiametric ray cells were observed only in the rays more than four cells height (Fig. 57D). In tangential view, terminal cells in these rays were vertically upright while the others were more or less isodiametric (Fig. 57D). In transverse view also the ray cells in the limit of growth ring (latewood) were more or less isodiametric while the others (early wood) were upright (Fig. 57C).

Secondary phloem was composed of sieve tube elements, companion cells, axial and ray parenchyma cells. Length and width of interxylary sieve tube elements were measured about 338 to 352 μm and 23 to 27 μm, respectively, while it was 315 to 332 μm and 25 to 28 μm, respectively, for normal external phloem. Similar to the xylem, phloem rays were mostly uniseriate while biseriate rays were observed occasionally. Each sieve tube element was accompanied by a single companion cell and was characterized by simple sieve plates with well-developed sieve areas on the lateral walls. Older phloem elements formed earlier by the previous successive rings showed heavy accumulation of callose followed by its obliteration while sieve tube elements adjacent to each successive ring of the cambium is functional and found free from heavy accumulation of callose.