Polish Botanical Journal 53(1): 5–13, 2008

STRUCTURE AND DEVELOPMENT OF INTER- AND INTRAXYLARY PHLOEM IN *LEPTADENIA RETICULATA* (ASCLEPIADACEAE)

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Abstract. Observations by light microscopy showed that in *Leptadenia reticulata* (Retz.) Wight & Arn. (Asclepiadaceae) the internal phloem differentiates centripetally as discrete strands simultaneously with the centrifugal differentiation of the external protophloem in the first visible internode of the stem. Internal phloem strands are discrete and develop from the marginal semi-mature parenchyma cells of the pith. Thereafter protoxylem elements are differentiated from the procambium between the internal and external protophloem. As seen in transverse section, external phloem differentiates as a continuous band over time. In mature stems, internal protophloem becomes nonfunctional through heavy accumulation of callose and disappears. New internal phloem strands differentiate from the marginal pith cells that replaced the nonfunctional internal protophloem. As secondary growth progresses further, certain segments of the vascular cambium temporarily lose their normal activity and begin to differentiate secondary phloem both centripetally and centrifugally. Soon afterwards it resumes its normal activity and begins producing thick-walled lignified secondary xylem centripetally. This process is repeated several times, giving rise to a number of islands of thin-walled parenchyma along with sieve tube elements embedded in the thick-walled secondary xylem. As the stem thickens, bands of included phloem became tangentially and radially wider than the islands formed in the beginning. In some samples, arcs of internal cambium eventually differentiated on the outer boundary of internal phloem strands that differentiated unidirectionally, producing only phloem elements centripetally.

Key words: included phloem, inter- and intraxylary phloem, *Leptadenia*

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INTRODUCTION

Interxylary phloem is the term applied to phloem produced internally by a single cambium. It can take different forms: one or two sieve tubes as in *Canavalia* DC. (Rajput 2003), larger bands as in *Salvadora*, confluent axial parenchyma as in *Combretum* Loefl., or larger phloem strands wider tangentially than radially as in many Onagraceae (Carlquist 1988). Although inter- and intraxylary phloem is known to occur in a number of dicotyledonous families, it is comparatively rare and restricted to a tiny portion of dicotyledons. Moreover, our understanding of interxylary phloem has been hampered by the fact that most of the wood samples are obtained in a dried condition and the usual treatment prior to sectioning usually does not adequately restore the phloem.

Maheshwari (1935) called attention to the presence of interxylary phloem in species of *Leptadenia* R. Br. No mention of it was made by Sabnis (1921), Blatter *et al*. (1929) and Sayeedud-Din and Suxena (1940). Later, Singh (1943) studied the distribution of interxylary and intraxylary phloem in two species of *Leptadenia* (*L. reticulata* (Retz.) Wight & Arn. and *L. spartium* Wight) and compared it with *Strychnos nux-vomica* L., without paying much attention to its development except for reporting that the internal phloem is differentiated from the marginal pith parenchyma. Apart from this work, no information is available on the structure and development of intraxylary and interxylary phloem of *Leptadenia*. The present investigation was intended to elucidate the origin, structure and development of intraxylary and interxylary phloem in *Leptadenia reticulata* (Retz.) Wight & Arn.

Materials and methods

Samples of *Leptadenia reticulata*, from very young shoot to mature stem (*ca* 20 mm thick), were collected from ten different plants growing on the Maharaja Sayajirao University campus at Baroda. Samples at various developmental stages were collected and fixed in FAA: for primary growth, starting from the branch tip to one meter away from the tip; and for secondary growth, mature branches and stems measuring 1.5 to 2 cm in diameter. Suitably trimmed samples starting from the first visible internode to the 25th internode were dehydrated in a TBA series and embedded in paraffin after infiltration (Berlyn & Miksche 1976). Serial transverse, tangential and radial longitudinal sections of 12–15 μm thick were cut with a rotary microtome; sections of mature stem and branches were cut with a sliding microtome. Sections were stained with safranin-fast green (Johanson 1940) and with a tannic acid-ferric chloride lacmoide combination (Cheadle *et al*. 1953). Length and width of sieve tube elements were measured directly from tangential longitudinal sections. For included phloem, length and width of sieve tube elements were measured from phloem islands adjacent to the cambium. Means and standard deviations were calculated from 100 random measurements. Important results were microphotographed with a Leica trinocular research microscope.

Results

Anatomy of young stem

In young stems of *Leptadenia*, the epidermis consists of a single compact layer of isodiametric, thin-walled parenchyma cells. A thin layer of cuticle covers the epidermis, while a 2–3-layered hypodermis composed of isodiametric thin-walled parenchyma cells differentiate beneath the epidermis. The bulk of the cortex consists of thin-walled parenchyma in which several immature secretary ducts are distributed randomly. However, the inner layer of cortex forms a discontinuous cylinder of thick-walled, lignified perivascular fibers. An endodermis or pericycle is indistinct in the stem. The pith consists of small thin-walled, isodiametric parenchyma cells and secretary ducts distributed randomly in the outer zone of the pith. Later on, as secondary growth proceeds, they also develop 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 in the formation of included phloem 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 differentiates from semimature pith cells. Its differentiation starts simultaneously and precedes that of the protoxylem. Prior to the complete development and lignification of protoxylem derivatives, well-developed discrete strands of external and internal phloem occur in the second visible internode (Fig. 1A & B). Gradually the bands of procambium become distinct, frequently interrupted by alternate bands of parenchyma cells (Fig. 1B & C). Procambial initials undergo tangential divisions to form radial rows of procambial cells and recent derivatives of protoxylem (Fig. 1C). The formation of procambial initials and protoxylem elements separates the external and internal phloem (Fig. 1D). At this stage the stem is composed of numerous collateral bundles consisting of external protophloem, protoxylem and internal protophloem. The protoxylem is composed of xylem parenchyma and a few elongated vessel members. These primary xylem elements possess annular and helical thickenings with a 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 undergo tangential divisions, and form new derivatives (Fig. 1D). At this stage a narrow zone of actively dividing procambial initials internal to the external protophloem forms a cambium-like meristem (i.e., metacambium). Tangential divisions of this meristem subsequently produce metaphloem centrifugally and metaxylem centripetally (Fig. 1D). The metaphloem consists of sieve tubes, companion cells and small phloem parenchyma cells. The sieve tube members are relatively narrow and possess simple sieve plates on their end walls, which lie slightly oblique to

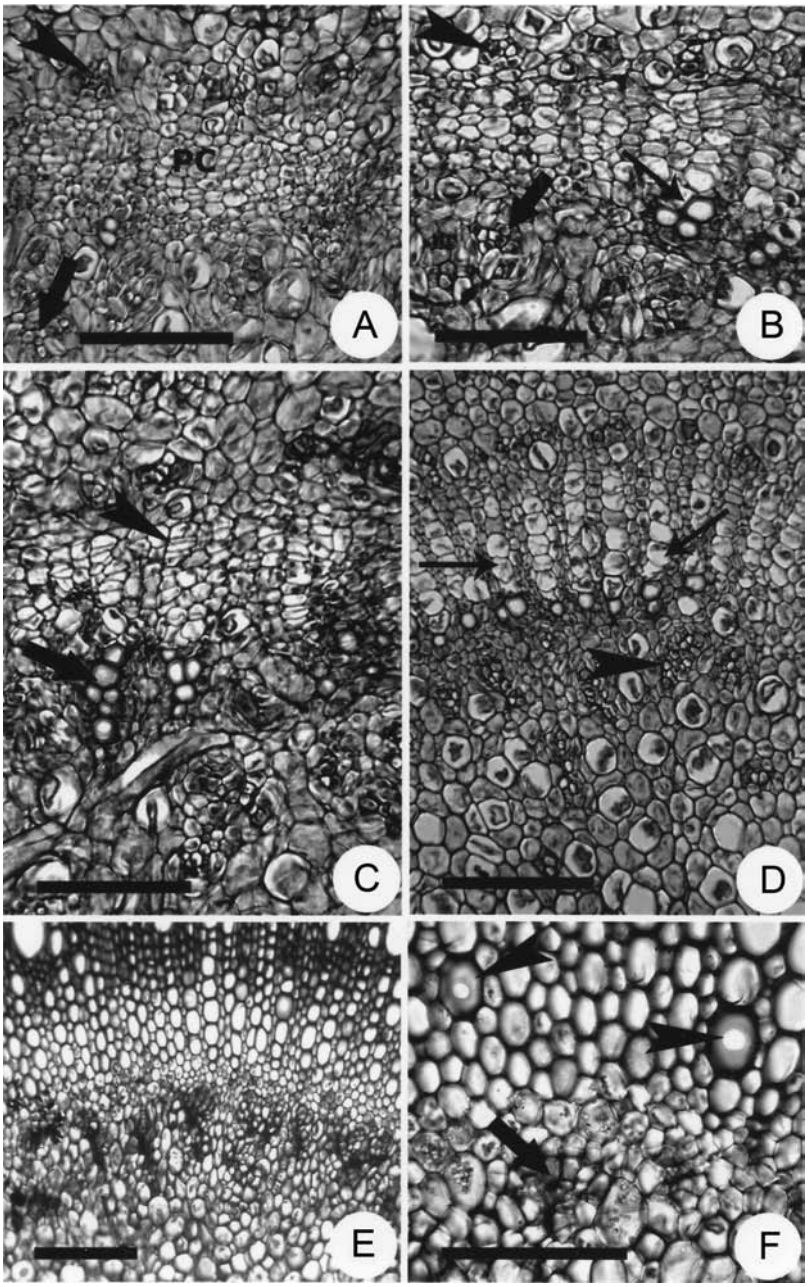

Fig. 1. Transverse section of young stem of *Leptadenia reticulata* (Retz.) Wight & Arn., showing origin of internal phloem. A – young stem with procambial tissue. Second internode showing well-differentiated discrete strands of external (arrowhead) and internal (arrow) phloem; B – strands of external (arrowhead) and internal (arrow) phloem in the next node, showing recently differentiated protoxylem elements (small arrow). Note the tangential division and radial arrangement of procambial cells; C – radially arranged procambial cells (arrowhead) separating external and internal phloem strands. Arrow shows recently formed protoxylem elements; D – metacambium with differentiating metaxylem elements (arrows). Arrowhead indicates internal phloem strands; E – mature stem, showing distribution of internal phloem beneath the protoxylem; F – enlarged view of mature stem, showing small perforation plates on suddenly tapering end walls (arrowheads). Arrow indicates obliterated internal protophloem. PC – procambium. Scale bars: 75 μm (A–D, F), 100 μm (E).

transverse. The metaxylem elements are arranged in radial rows (Fig. 1D) that are separated by uni- to biseriate parenchyma cells. Newly formed metaxylem elements consist of tracheids, fiber tracheids, parenchyma cells and vessels. The first-formed metaxylem vessel members are narrow and possess helical or reticulate secondary thickenings. Their end walls taper suddenly, with a very small simple perforation plate on their transverse to slightly oblique end walls (Fig. 1E & F). The diameter of these perforation plates is almost half the diameter of the vessel member. Later-formed vessel members may be narrower, with a simple perforation, and possess small, opposite or alternate on lateral walls.

FORMATION OF INTERNAL CAMBIUM

In mature stems, when secondary growth is in progress, older nonfunctional internal phloem strands are replaced by the addition of new elements from adjacent parenchyma cells (Fig. 2A & B). As the internal phloem become 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. 2C). In some samples, small arcs/segments of internal cambium are formed on the outer side of the internal phloem strands (Fig. 2D). This internal cambium arises from fully matured pith parenchyma cells by repeated tangential divisions. Parenchyma cells giving rise to the internal cambium are located along the outer margin of internal phloem strands. The internal cambium is unidirectional in nature and cuts off only phloem elements centripetally. Structurally this phloem is more or less similar to that of other internal phloem cells; no differentiation of any xylem derivatives from this cambium was observed (Fig. 2D).

SECONDARY GROWTH AND FORMATION OF INCLUDED PHLOEM

Like other dicotyledons, with the initiation of secondary growth, the cambium produces 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 lose their normal activity and begin to reproduce sieve tube elements and axial parenchyma cells internally (Fig. 3A). During differentiation, usually the cambium differentiates into parenchyma cells, which later on differentiate into sieve tube elements. Sometimes the fusiform cambial cells directly differentiate into sieve tube elements instead of xylem elements towards the inner side (Fig. 3B & C). This feature is not common. 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 differentiate 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 regain normal cambial activity and begin to produce normal thick-walled secondary xylem centripetally. Later, another segment of the cambium behaves in a similar manner. Such repeated behavior of the cambium leads to the formation of phloem islands along with parenchyma embedded in the secondary xylem, resulting in included phloem (Fig. 3A).

As the stem thickens, segments of cambium producing parenchyma cells and sieve tube elements also widen, forming larger bands of included phloem embedded in xylem. Sometimes these bands cover three of four adjacent rays, so that some of the rays pass through the phloem islands (Fig. 3D–F). In mature stems (i.e., 15 to 20 mm diameter), the sieve tube elements produced in the beginning of secondary growth become nonfunctional through heavy accumulation of callose. The nonfunctional phloem gradually collapses and disappears, leading to radial and tangential expansion of the adjacent parenchyma cells. Later on these parenchyma cells undergo periclinal divisions and differentiate into new sieve tube elements (Fig. 3F). Structurally the sieve tube elements of both normal and included

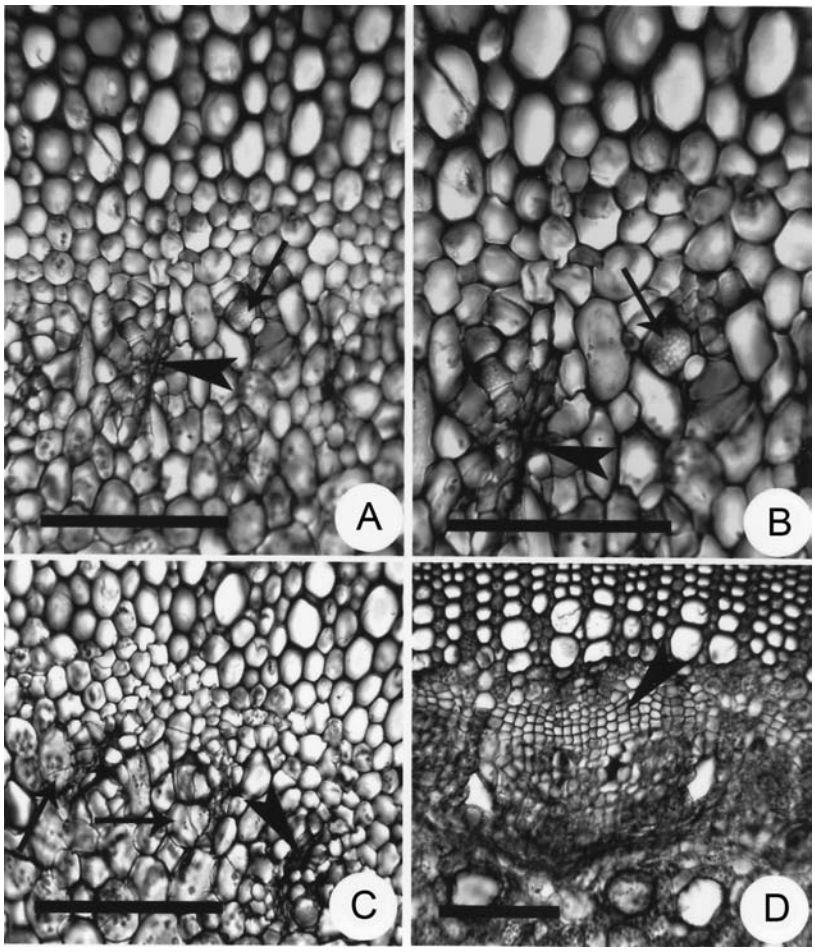

Fig. 2. Transverse section of mature stem of *Leptadenia reticulata* (Retz.) Wight & Arn., showing secondary internal phloem. A – obliteration of nonfunctional internal protophloem and newly formed sieve tube elements (arrow). Note parenchyma at upper left, showing recently formed tangential wall; B – enlarged view of Fig. 2A. Arrow indicates sieve tube elements with companion cell and obliterating protophloem (arrowhead); C – obliteration of protophloem (arrowhead) and enlargement of adjacent parenchyma followed by periclinal division (arrow); D – internal cambium (arrowhead) with functional internal phloem on its lower side. Scale bars: 75 µm (A–C), 100 µm (D).

phloem are more or less similar. The phloem is composed of sieve tube elements, companion cells and axial parenchyma cells, which are always found in contact with the adjacent ray cells (Fig. 3D & E). All three types of phloem differentiated in the stem differ in the length and width of the sieve tube elements. The sieve tube elements are longer and wider in included phloem (238 µm and 22 µm) and internal phloem (221 µm and 21 µm) than in external phloem (209 µm and 20 µm).

Discussion

The occurrence of internal phloem has been reported in several genera of dicotyledonous families. Metcalfe and Chalk (1950) listed 27 dicotyledonous families with internal phloem differentiation. Hartig (1854) reported internal phloem for the first time (in Scott & Brebner 1889) when working with *Cucurbita pepo* L. (Fukuda 1967). Nearly 95% of the genera with internal phloem differentiation belong to five orders (Contortales,

Cucurbitales, Tubiflorales, Myrtales and Thymeleales), and around 2/3 of the genera with internal phloem belong to the first three orders (Bonnemain 1969). Internal phloem also occurs in a number of fern species (Esau 1965).

Singh (1943) reported the occurrence and distribution pattern of inter- and intraxylary phloem in two species of *Leptadenia*, but a search of the available literature did not yield any detailed account of its developmental anatomy. Fukuda (1967) reported internal phloem in other members of Ascepiadaceae, such as *Cynanchum*, *Marsdenia* and *Tylophora*. Differentiation of internal phloem in *Leptadenia* occurs immediately adjacent to the apical portion, that is, in the first visible internode, prior to the differentiation of protoxylem. The formation of internal phloem prior to the differentiation of protoxylem led Herail (1885) and Lamounette (1890) to question the bicollateral nature of such a vascular arrangement. In the present study, internal phloem was found to differentiate from the marginal pith cell and not from the procambium responsible for the formation of normal outer protophloem. Various workers including Worsdell (1915), Artschwager (1918) and Woodcock (1935) have referred to such intervening tissue as perimedullary tissue, from which internal phloem differentiates in younger internodes.

As mentioned earlier, the internal phloem of *Leptadenia* differentiates from marginal parenchyma cells. The tissue separating the vascular cylinder from internal phloem strands has a different appearance, and the cells surrounding internal phloem strands are larger in diameter. Additional evidence indicating their origin from pith cells is evident from Figure 1A–C: some of the internal phloem strands are quite far from the phloem strands adjacent to the pith margin. Additional internal phloem strands may be produced later on by divisions in columns of vacuolated pith cells. A similar feature has been reported in marglobe tomato (Venning 1949) and in some members of Asclepiadaceae (Fukuda 1967). However, Handa (1936) reported that internal phloem in *Marsdenia* has its origin in the same procambium as the normal external phloem but that there are several medullary strands produced by division of pith cells. Differentiation of internal phloem may occur before, after, or simultaneously with the origin of external normal phloem. Artschwager (1918) reported earlier development of internal phloem in Irish potato, while Mikesell and Schroeder (1984) recorded its origin after the differentiation of external phloem in *Pharbitis nil* (L.) Choisy. In the present study, internal phloem and normal external phloem was found to arise simultaneously. Similar findings have been reported in several plants such as *Strychnos* L. and *Apocynum* L. (Scott & Brebner 1889, 1891), *Cucurbita maxima* Ducesne (Faber 1904), *Convolvulus* L. (Kennedy & Crafts 1931), *Cucurbita pepo* L. (Whitting 1937) and *Solanum* L. (Sussex 1955). Fukuda (1967) noted simultaneous development of internal and external phloem in Asclepiadaceae.

Later in the development of internal phloem, when the secondary growth is in progress, the first-formed internal phloem becomes nonfunctional and additional new phloem elements produced by adjacent pith cells fill the cavity left by the disappearance of nonfunctional sieve tube elements. Interestingly, in some mature stem samples we also noted small cambial arcs (internal cambium) between the inner margin of protoxylem and the outer margin of internal phloem strands; these cambial arcs produced secondary internal phloem towards the center of the pith. Vesque (1875) discovered such cambium for the first time in Solanaceae, Asclepiadaceae and Apocynaceae; Petersen (1882) reported it in Myrtaceae and Lythraceae. Earlier works variously interpreted such internal cambium as, for example, false cambium (Vesque 1875), local cambium (Scott & Brebner 1989), unilateral cambium (Baranetzky 1900) and unidirectional cambium (Philipson & Ward 1965). In the present study we prefer the term 'internal cambium,' as it is directly concerned with the production of internal phloem. Its presence in some though not all the samples supports the observations of Singh (1943) in *Leptadenia*. Scott and Brebner (1891) reported the occurrence of internal cambium in *Periploca* L. (Asclepiadaceae), and Handa (1936) reported it in *Marsdenia* R. Br. of the same family. This internal cambium of *Periploca* and *Marsdenia* is said to produce both secondary xylem and secondary phloem.

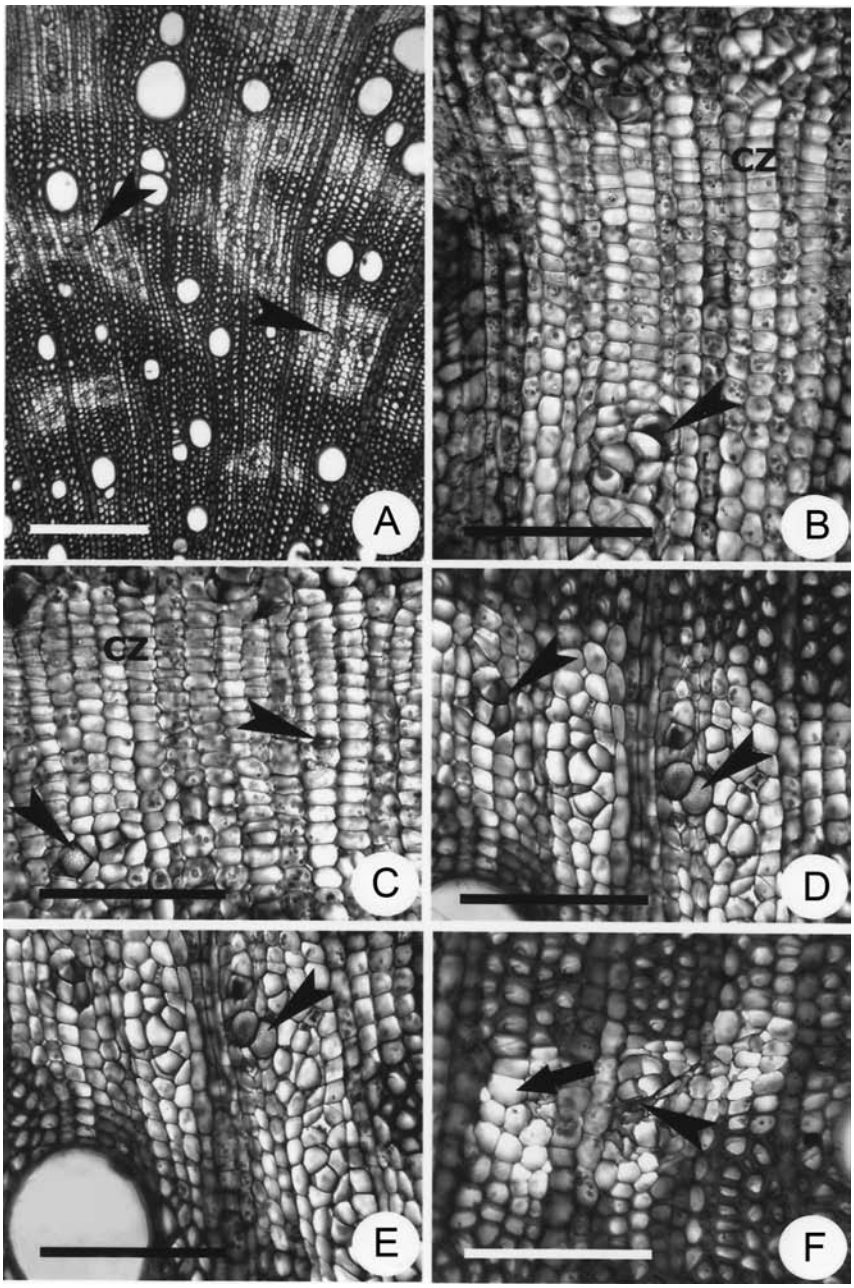

Fig. 3. Transverse section of mature stem of *Leptadenia reticulata* (Retz.) Wight & Arn., showing included phloem. A – distribution of included phloem in mature stem (arrowheads). Note the increase in size of included phloem islands from pith towards the periphery; B – newly differentiated sieve tube elements internal to and adjacent to the active cambium (arrowhead). Note the difference in sieve tube element diameter between external and included phloem; C – differentiating sieve tube elements internal to and adjacent to the active cambium (arrowheads); D – included phloem island surrounded with thick-walled lignified xylem elements. Arrowheads indicate fully differentiated sieve tube elements; E – distribution of included phloem in mature stem. Arrowhead indicates fully differentiated sieve tube elements; F – obliteration of nonfunctional included phloem (arrowhead). Note the radial and tangential expansion of adjacent parenchyma cells (arrow). CZ – cambial zone. Scale bars = 100 μm.

In our samples of *Leptadenia* we observed only differentiation of phloem, and not xylem.

The term 'interxylary phloem' should be restricted to the product of a single cambium produced internally as strands of (interxylary) phloem embedded within bands of axial parenchyma surrounded by secondary xylem (Carlquist 2002). The term 'included' is a misnomer in the case of dicotyledons with successive cambia, because conjunctive tissue in those species is formed either as background tissue or as bands between one vascular band and another, and thus by definition is not 'included' within the secondary xylem of species with successive cambia (Carlquist 2002, 2004).

In *Leptadenia*, at sites of secondary xylem the cambium forms secondary phloem along with thin-walled parenchyma cells. This is a temporary phase, however, and it soon resumes its normal activity producing the usual secondary xylem elements. This process is repeated several times, giving rise to a number of thin-walled tissues embedded in the thick-walled cells of the wood. Singh (1943) reported a similar pattern of phloem island formation in *Leptadenia*. He correlated the formation of included phloem in *Leptadenia* with that in *Strychnos*, but the origin of included phloem is quite different in the latter. In *Leptadenia* secondary phloem originates on both the outher and inner sides of the single normal cambium or from the parenchyma cells that yield additional sieve tube elements and companion cells towards the inside of the stem. Carlquist (2002) called the parenchyma that produces included phloem in *Salvadora* Garcin *ex* L. 'residual meristem.' Sieve tube elements and companion cells are added continuously over time, and as the phloem islands are surrounded by thick-walled xylem elements there is no scope for expansion of newly formed elements; this results in crushing of older and nonfunctional elements.

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

ACKNOWLEDGEMENTS. We thank the Department of Biotechnology of the Ministry of Science and Technology of India for financial support, and the anonymous reviewer for valuable remarks on the manuscript.

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Received 10 January 2008