STRUCTURE AND DEVELOPMENT OF INTERNAL PHLOEM IN 
SOLANUM PSEUDOCAPSICUM (SOLANACEAE)

Vidya S. Patil, Rina D. Koyani, Gaurav V. Sanghvi and Kishore S. Rajput*

Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India
*Corresponding author; e-mail: ks.rajput15@yahoo.com

ABSTRACT

The development of internal phloem in the Jerusalem cherry, Solanum pseudocapsicum L. (Solanaceae), was studied in young and mature stems. The early presence of primary internal phloem is succeeded by the development of secondary internal phloem from an internal cambium situated between the protoxylem and primary internal phloem. In the second and third visible internodes of the young stem, procambial derivatives begin to differentiate as discrete strands of internal protophloem in a perimedullary position prior to the differentiation of protoxylem and external protophloem. In 6–8 mm diameter stems, sieve elements of the internal phloem become non-conducting, begin to collapse, and undergo obliteration. In 15–20 mm diameter stems internal cambium is initiated from the parenchyma cells situated between the protoxylem and primary internal phloem. The development of internal phloem and an internal cambium in S. pseudocapsicum is compared with that in other taxa. There seems to be a gradual variation in the origin of an internal cambium from either remnants of the procambium or dedifferentiation of peripheral pith cells across dicotyledons with an internal cambium.

Keywords: Intraxylary phloem, internal cambium, secondary phloem, cambial variant, Jerusalem cherry.

INTRODUCTION

Phloem strands that form between the pith and the primary xylem in certain plant families are termed internal or intraxylary phloem. The occurrence of internal phloem in Cucurbita pepo was reported about one and a half century ago by Hartig (1854), subsequently Vesque (1875) reported its presence in the Solanaceae and the Apocynaceae. Since then, numerous studies reported the presence of internal phloem in different species belonging to different families of eudicots (Scott & Brebner 1889; Solereader 1908; Esau 1938, 1969; Singh 1943; Pamela & Wilson 1961; Fukuda 1967; Bonnemain 1968, 1972; Zamski & Tsivion 1977; Kuo & Pate 1981; Zozimo et al. 2011). Though presence of internal phloem is documented in about 30 families (Metcalfe & Chalk 1983), there are very few species that develop internal cambium at the pith margin (Fukuda 1967).
Kuo and Pate (1981) observed considerable variation in the structure and origin of internal phloem. According to them the number of sieve elements produced in the strands of internal phloem also varies from species to species (Esau 1969; Bonnemain 1972). Esau (1969) provided a detailed description on the origin and differentiation of internal phloem in *Nicotiana* (Solanaceae). Perusal of the literature indicates that internal phloem may develop prior to (Baranetzky 1900; Zozimo et al. 2011), after, or concurrent with primary vasculature (Esau 1969; Bonnemain 1972; Kuo & Pate 1981). Earlier researchers demonstrated the role of internal phloem in the translocation of photosynthates by using carbon isotopes (14C) in different species of the Solanaceae such as *Lycopersicon esculentum* L. (Bonnemain 1968, 1972), *Nicotiana tabacum* L. (Zamski & Tsivion 1977), *Nicandra physaloides* L. (Bonnemain 1980) and *Raphanus sativus* L., of the Brassicaceae (Fredon & Bonnemain 1970).

The family Solanaceae is characterized by the presence of internal phloem (Pamela & Wilson 1961; Fukuda 1967; Esau 1969; Metcalfe & Chalk 1983) and its origin is generally considered as primary but additional sieve elements may develop following de-differentiation of parenchyma cells (Esau 1938; Patil et al. 2009). The irregular longitudinal divisions in promeristem cells give rise to procambial strands in which the inner cells differentiate into phloem elements (Baranetzky 1900; Esau 1938). In contrast, the marginal pith cells in *Leptadenia* (Singh 1943; Patil et al. 2008) and *Ipomoea hederifolia* L. (Patil et al. 2009) become meristematic as secondary growth progresses and develop into an internal cambium. Pamela and Wilson (1961) have also reported the development of internal cambium in wounded stems of certain Solanaceae species.

The occurrence of internal phloem is considered characteristic for the Solanaceae but development of internal cambium is rare in this family (Fukuda 1967). Our earlier study reported the occurrence of internal cambium in *Solanum pseudocapsicum* while describing its general anatomy and the pharmacognosy (Sanghvi et al. 2011). Therefore, the present study focuses on the origin, structure and development of internal phloem as well as internal cambium in *Solanum pseudocapsicum* L.

**MATERIALS AND METHODS**

Samples of *Solanum pseudocapsicum* L., from very young shoots (apical portion) to mature stems (20 mm diameter), were collected from 10 different plants growing in the M.S. University Campus at Baroda (India). For the primary growth, samples for various developmental stages starting from the shoot tip up to the 15th internode were collected. For secondary growth analysis, samples were collected from the middle (40–45 cm from ground level) and basal (10 cm above ground level) part of the stem (about 15 to 18 mm in diameter). Immediately after collection they were fixed in FAA (Berlyn & Miksche 1976). After 12 hrs of fixation, samples were transferred to 70% alcohol for further storage and processing. Suitably trimmed samples starting from first visible internode up to the 15th internode were dehydrated in tertiary butyl alcohol (TBA) series, infiltrated in para TBA (paraffin : TBA, 1 : 1) and embedded in paraffin (Johansen 1940). Serial transverse, tangential and radial longitudinal sections of 12–15 µm thickness were obtained with the help of a Leica rotary microtome while thick
stems and branches were sectioned with the aid of a sliding microtome. The sections were stained with safranine-fast green (Johansen 1940) or tannic acid-ferric chloride-lacmoid (Cheadle et al. 1953). Length and width of sieve tube elements were obtained directly from the tangential longitudinal sections. In case of internal phloem, length and width of sieve tube elements were obtained from the phloem islands adjacent to the primary xylem. Thirty measurements at random were considered to obtain the mean.

RESULTS

Anatomy of young stems

In the young stems, the epidermis consists of a single compact layer of isodiametric and thin-walled parenchyma cells (Fig. 1A) covered with a thin layer of cuticle. The cortex consists of parenchyma cells that showed heavy accumulation of starch grains in 12–13 mm diameter stems (Fig. 1B). Isolated sclereids occur in the periphery of the cortex (Fig. 1C) while isolated or groups of 2–4 pericyclic fibres are observed on the inner margin of the cortex (Fig. 1D). Pith cells also showed heavy accumulation of starch grains and raphide bundles (Fig. 1E, F). Some of the cells in the cortex are filled with crystals (Fig. 1B). After a short period of secondary growth, isolated or groups of 2–4 sclerenchymatous cells (referred here as internal phloem fibres) differentiate on the inner margin of the internal phloem (Fig. 1F, 2B).

The internal phloem may be separated into two types, i) primary internal phloem, and ii) secondary internal phloem.

Development of primary internal phloem

Differentiation of internal protophloem precedes that of regularly positioned external protophloem. During the differentiation, cells situated below the promeristem (i.e., immediate derivatives of promeristem) give rise to protoderm, procambium and the ground tissue (Fig. 1A). Cortical and pith cells are much larger than the procambial cells (Fig. 2A, C, D). In the second internode, procambial derivatives situated on the inner margin of procambium differentiate into internal phloem strands (Fig. 2C, D). On the other hand, the origin of regularly positioned external protophloem is observed only after the initiation of protoxylem differentiation (Fig. 2E). In this internode, strands of 8–10 internal phloem cells may be seen towards the pith while external protophloem strands only number 2–4 cells (Fig. 2E). Prior to complete differentiation and lignification of protoxylem, well established discrete strands of external and internal phloem are visible in the third internode. Procambial cells at this stage divide repeatedly and establish a radial cell arrangement (Fig. 2E).

Procambial cells in the 4th internode undergo tangential divisions and give rise to radial rows of protoxylem internally and normal protophloem externally. At this stage, the vascular system is composed of regularly positioned protophloem external to procambium and protoxylem on the inner side while strands of internal protophloem situated inside to the protoxylem are separated by parenchyma cells. The protoxylem is composed of vessel elements and few xylem parenchyma cells. Vessel elements are characterized by annular and helical secondary walls (Fig. 2F) with simple perforation
Figure 1. Transverse (A–D), radial (E) and tangential longitudinal (F) view of young stem of Solanum pseudocapsicum. – A: Structure of very young stem composed of single-layered epidermis, few-layered cortex, procambium (arrow) and differentiating internal phloem (arrowhead). P = pith. – B: Cortical parenchyma in thick stems showing deposition of starch grains (arrows) and crystal sand (arrowhead). – C: Stone cells (arrowheads) in the cortex of 17-mm-thick stem. – D: Young stem showing pericyclic fibres (arrowheads). – E: Pith cells showing calcium oxalate needles in raphide bundles in the pith cells. – F: Longitudinal section. Arrowheads indicate protoxylem element with helical secondary wall and internal phloem fibres (arrows). — Scale bars in A–E = 100 µm; in F = 150 µm.
Figure 2. Transverse (A–E) and radial longitudinal (F) view of young stem showing procambium, external and internal protophloem. – A: Structure of young stem composed of single cell layered epidermis, parenchymatous cortex (*) and internal phloem (arrowheads). – B: Relatively thick stem showing internal phloem fibres on the inner margin of crushed internal phloem (arrowheads). – C: Internal protophloem (arrowhead). Note that normal external protophloem is still absent. – D: Normal external protophloem (arrowheads) occurs only after the initiation of protoxylem differentiation (arrows). – E: Procambium showing radial arrangement of the cells (arrowhead). Arrow indicates sieve elements. – F: Longitudinal section. Arrowheads indicate protoxylem element with helical to annular secondary wall. — Scale bars in A–D & F = 100 µm; in E = 150 µm.
plates. In the 6th internode, subsequent tangential divisions occur in the interfascicular region and all the vascular bundles are joined to form a complete cylinder. The vascular cylinder is composed of metaxylem and metaphloem along with protoxylem and protophloem. The metaxylem is composed of vessels, tracheids, and parenchyma cells. At this stage patches of internal phloem become distinct and the thin-walled procambial derivatives are clearly visible between the first formed protoxylem elements and in-

Figure 3. Transverse (A–D) view of young stem showing origin of cambium and development of internal protophloem. – A: Enlarged view of cambium from the 6th internode. Note the peripheral procambial derivatives situated inwards from the protoxylem that will act as the site for origin of the internal cambium (arrowheads). – B: Stem portion at the 10th internode showing external regular protophloem, internal phloem and internal phloem fibres (arrowheads). – C: Primary internal phloem formed prior to development of internal cambium (arrowheads). Note the crushed internal protophloem (arrow). – D: Thick stem showing internal cambium (arrowheads). Also note the radially elongated ray-like parenchyma cells (arrows). — Scale bars = 100 μm.
Patil et al. – Internal phloem in Solanum

Internal protophloem (Fig. 3A). These cells serve as the site for the origin of the internal cambium. In the 10th internode, internal phloem is composed of sieve tubes, companion cells and parenchyma. A considerable amount of internal phloem has accumulated prior to development of the internal cambium in all the samples studied. Concomitant with internal protophloem development, some of the procambial derivatives also differentiate into isolated internal phloem fibres (Fig. 3B).

**Development of secondary internal phloem**

Secondary internal phloem develops from an internal cambium (Fig. 3C, D). Prior to the origin of the internal cambium, earlier formed sieve elements show heavy accumulation of callose (Fig. 4A, B) and begin to collapse (Fig. 3C). Concurrently parenchyma cells situated inside to primary xylem undergo dedifferentiation and form small arcs of internal cambium on the outer face of the internal phloem strands (Fig. 3D). Structurally the phloem produced from the internal cambium is more or less similar to the external phloem and is composed of sieve tubes, companion cells, and axial parenchyma cells. Some radially elongated cells, appearing like ray cells are also observed in the internal phloem (Fig. 3D). Sieve tube elements measure about 262 to 280 µm in length and 19 to 31 µm in width. Additional secondary internal phloem fibres differentiate on the inner margin of the secondary internal phloem. The detailed structure of secondary xylem is described elsewhere (Sanghvi *et al.* 2011).

Figure 4. Transverse (A) and radial longitudinal (B) view of *Solanum pseudocapsicum* stems showing internal phloem and internal cambium. – A: Internal phloem (sieve elements) showing callose in some of the sieve elements (arrowheads). – B: Sieve tube elements of internal phloem showing callose (arrowhead) on sieve plates of non-functional sieve elements. Arrow shows functional sieve tube element with sieve plate. – P = pith, * = crushed sieve elements. — Scale bars = 50 µm.
DISCUSSION

Internal phloem occurs in the stems of all Solanaceous plants (Esau 1938, 1969; Pamela & Wilson 1961; Fukuda 1967; Bonnemain 1972; Kuo & Pate 1981; Metcalfe & Chalk 1983) and remains in close vicinity of the protoxylem. However, internal phloem is usually separated from protoxylem by a few parenchyma cell layers (Fukuda 1967). Ontogenetically, internal phloem is regarded as primary in origin (Worsdell 1915; Artschwager 1918; Kennedy & Crafts 1931; Woodcock 1935; Esau 1938; Fukuda 1967, Mikesell & Schroeder 1984). It arises at the inner margin of the procambial ring by irregular longitudinal divisions in cells situated on the inner side of the procambium and differentiates centrifugally into phloem elements (Baranetzky 1900; Esau 1938). However, in the present study, these phloem elements subsequently increase in number by differentiation of adjacent parenchyma cells into internal phloem elements. A similar origin of additional sieve elements from the adjacent parenchyma cells have also been reported by Pamela and Wilson (1961) in Solanaceae and Leptadenia (Patil & Rajput 2008).

Internal phloem development has hitherto been investigated from various angles by many investigators and is still open to debate (Fukuda 1967). There is a controversy about the origin of internal phloem. According to earlier researchers, it may develop from various tissues such as: i) procambium (Scott & Brebner 1889; Baranetzky 1900; Kennedy & Crafts 1931; Zozimo et al. 2011), ii) procambial derivatives (Fukuda 1967; Mikesell & Schroeder 1984; Patil et al. 2009), or iii) dedifferentiation of the marginal pith cells (Esau 1938; Singh 1943; Patil & Rajput 2008; Patil et al. 2009). In the present study internal protophloem originates from procambial derivatives while secondary internal phloem develops from an internal cambium. There are reports that internal phloem also develops from peripheral pith cells in a number of other families (Lamounette 1890; Handa 1936; Fukuda 1967).

Differentiation of internal phloem may occur before, after, or simultaneously with the origin of external normal phloem (Baranetzky 1900; Esau 1938, 1969; Singh 1943; Fukuda 1967; Bonnemain 1972; Kuo & Pate 1981; Patil & Rajput 2008; Patil et al. 2009; Zozimo et al. 2011). Simultaneous origin of internal phloem and normal external phloem has been reported in several plants such as Strychnos L. and Apocynum L. (Scott & Brebner 1889, 1891), Cucurbita pepo Duquesne (von Faber 1904), Convolvulus (Kennedy & Crafts 1931), Cucurbita maxima (Whiting 1937) and even in some species of Solanum (Sussex 1955). However, in the present study, internal phloem arises before the development of normal external phloem. Similar observations are also reported in Irish potato by Artschwager (1918), in Quisqualis indica by Baranetzky (1900) and in Combretum rotundifolium by Zozimo et al. (2011). In contrast, Mikesell and Schroeder (1984) documented its origin after the differentiation of external phloem in Pharbitis nil of the Convolvulaceae.

In Solanum pseudocapsicum with the advancement of secondary growth, the first formed internal phloem deposits callose and begins to collapse, leading to the obliteration of sieve elements (non-conducting phloem elements). Concomitant to obliteration, development of additional sieve elements takes place from the parenchyma cells
located on the inner side of the protoxylem and from an internal cambium. Similar events of internal phloem elements are also recorded in *Leptadenia* (Singh 1943; Patil & Rajput 2008). In 15–20 mm (diameter) thick stems, small arcs of internal cambium originate between the inner margin of protoxylem and outer margin of internal phloem. In *Solanum lycatum* internal cambium originates in a similar way as observed in the present study (Fukuda 1967). Internal cambium has been interpreted variously by different workers such as: false cambium (Vesque 1875), unilateral cambium (Baranetzky 1900), unidirectional cambium (Philipson & Ward 1965), local cambium (Scott & Brebner 1989) or internal cambium (Fukuda 1967; Patil & Rajput 2008; Patil et al. 2009; Zozimo et al. 2011) or phloem cambium (cf. Fukuda 1967).

Different physiological functions have been ascribed to the presence of internal phloem (Pamela & Wilson 1961; Fukuda 1967; Bonnemain 1968, 1972, 1980; Esau 1969; Zamski & Tsivion 1977; Fredon & Bonnemain 1970; Kuo & Pate 1981; Mikesell & Schroeder 1984; Patil & Rajput 2008). Internal phloem is said to play an important role in the maintenance of apical dominance and localized redistribution of photosynthates in the stem and branches (Botha et al. 1975; Zamski & Tsivion 1977; Kuo & Pate 1981; Zozimo et al. 2011) while Bonnemain (1968, 1969) considered it to play a crucial role in long-distance translocation of photosynthates. On the other hand, phloem elements present on the inner margin of bicollateral vascular bundles of Cucurbitaceae are involved in transport of macromolecules, proteins, amino acids and a wide range of unidentified secondary metabolites (Turgeon & Oparka 2010; Zhang et al. 2010). A similar function may be performed by the internal phloem in *Solanum pseudocapsicum*, but further studies are needed to establish this.

**CONCLUSION**

In *Solanum pseudocapsicum* internal protophloem develops from procambium prior to the development of normally positioned external protophloem. Additional secondary internal phloem develops from parenchyma cells situated inside to protoxylem and an internal cambium.

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