Polish Botanical Journal 54(1): 113–121, 2009

STRUCTURE AND DEVELOPMENT OF SECONDARY THICKENING MERISTEM IN *MIRABILIS JALAPA* (NYCTAGINACEAE)

KISHORE S. RAJPUT, VIDYA S. PATIL & KAILASH K. KAPADNE

Abstract. The structure and development of vascular cambium and its derivatives were studied in normal and experimentally injured stems of *Mirabilis jalapa* L. (Nyctaginaceae). In normal stems the cambium was semi-storied and composed exclusively of fusiform cambial cells with no rays. Several collateral vascular bundles were joined by interfascicular cambium and formed a complete ring of activity. After a definite period a small segment of cambium that produced conducting elements of xylem and phloem ceased to divide. A new segment of cambium was developed from the parenchyma cells outside the phloem of previous cambium. This newly formed cambial segment replaced the nonfunctional segment by joining with other functional segments to form a continuous ring. Each successive segment of cambium followed a similar pattern of development. Functionally the cambium was bidirectional, producing both xylem and phloem on opposite sides, but the rate of cell division towards phloem was very slow, thus making the cambium appear functionally unidirectional. Functional sieve elements were observed in all the phloem islands; nonfunctional sieve elements showed heavy accumulation of callose. Experimentally we tried to induce the formation of rays by injuring the cambium, but instead of forming wound meristem it formed cork cambium around the wounds. The meristematic segments present between the xylem and phloem of medullary bundles also remained active even at the senescent stage.

Key words: cambium, rayless xylem, medullary bundles, *Mirabilis*

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INTRODUCTION

Mirabilis jalapa L. (Nyctaginaceae), commonly known as Four O'clock, is an annual ornamental herb with dichotomous branching. The nodes are swollen, with relatively narrow internodes. In June, with the arrival of rains, new saplings develop from seeds or from the underground rhizome of previously growing plants. It completes its vegetative and reproductive growth by March-April. At the senescent stage, each internode starts to detach from the nodal region from the apical portion and spreads gradually towards the base, leaving an underground rhizome. The rhizome is soft and parenchymatous, full of starch.

Secondary plant tissues in Centrospermae have attracted the attention of various investigators (Yarrow & Popham 1981; Lotova & Timonin 1985; Timonin 1988; Lev-Yadun & Aloni 1995; Rajput & Rao 1998; Rao & Rajput 1998, 2003). Although much attention has been paid to studying the manner in which secondary thickening, development of medullary bundles, and development of included phloem take place, the source of these cambia and the way in which they act have been subject to diverse and controversial interpretations. Several theories have been proposed to describe the polycambial secondary thickening and its significance (Iljin 1950; Balfour 1965; Mikesell & Popham 1976; Yarrow & Popham 1981). As a typical example of Nyctaginaceae, secondary thickening has been studied in *Mirabilis* and *Boerhaavia* from time to time (Maheshwari 1930; Joshi 1937; Mikesell & Popham 1976). More recently, Carlquist (2004, 2007) also studied the pattern of secondary growth and formation of successive cambia in Nyctaginaceae and concluded that there are two kinds of meristems operative in axes of Nyctaginaceae: master cambium and vascular cambium. According to him, master cambium exists in the form of a complete ring, while vascular cambium originates from it to its inner face and usually these cambia are rayless.

In our earlier study of different species of *Boerhaavia* we reported the absence of rays in xylem and phloem (Rajput & Rao 1998). Since *Mirabilis* also possesses rayless cambium, we were interested in knowing whether injury can induce rays in such rayless cambium. Moreover, the vascular system of *Mirabilis* shows two features of special interest: (1) the development of medullary bundles from the procambial strand below the apex, and (2) the mode of secondary growth by which the stem increases in thickness. Maheshwari (1930) made detailed studies of the origin and development of the medullary bundles and cambial cylinder in *Boerhaavia diffusa*. According to him, the structure and development of medullary bundles in *Mirabilis* resemble those in *Boerhaavia*, but there are no published data available on the development of cambial derivatives, the rayless nature of the xylem, and the functional status of meristem present between the xylem and phloem of medullary bundles at the senescent stage of the plants. Here we report the rayless nature of the cambium, the response of the cambium to injury, and the development of secondary xylem and phloem in *Mirabilis jalapa* L. (Nyctaginaceae).

MATERIALS AND METHODS

Four to six stem pieces measuring 8–30 mm in diameter and 40–60 mm in length were collected from ten plants of *Mirabilis jalapa* L., and fixed immediately in FAA (Berlyn & Miksche 1976). All these samples were collected from plants cultivated in the botanical garden of the M. S. University of Baroda (central Gujarat), India. Some of the plants 8–10 mm in diameter (40–45 days old) were wounded with vertical, oblique and horizontal cuts with a razor blade to induce wound meristem. In another ten plants, 1 cm^2 patches of bark along with the cambium were removed with a sterile surgical blade and replaced in different orientations (e.g., 90°, 180°) or the same orientation and tied with thread as in grafting technique. These injured saplings were collected in December, four months from the date of injury. Transverse, tangential and radial longitudinal sections 12–15 μm thick were cut with a sliding microtome and stained with safranin-fast green (Johansen 1940) and with a combination of tannic acid, ferric chloride and lacmoide (Cheadle *et. al.* 1953). After passing through an ethanol-xylene series the sections were mounted in DPX.

Portions of xylem 1 mm wide bordering the cambial zone were macerated with Jeffrey's fluid (Berlyn & Miksche 1976) at 56–60°C for 24–36 h. Fusiform cambial cells and sieve elements were measured directly from the tangential longitudinal sections; dimensional details of vessel elements and xylem fibres were recorded from the macerated material. Average length of fusiform cambial cells, sieve tube elements, vessel elements and xylem fibres was calculated from 100 random measurements. Vessel frequency for a 0.5 mm^2 area of xylem in transverse section was measured with Qwin Plus Image Analyser (Leica Germany). Important results were microphotographed with a DM 2000 Leica trinocular research microscope.

RESULTS

STRUCTURE AND DEVELOPMENT OF CAMBIUM

The vascular cambium is semi-storied and composed exclusively of relatively short fusiform cambial cells (Fig. 1A, B). In some areas it shows a distinct storied pattern (Fig. 1B), and in others nonstoried. Fusiform cambial cells are 220–260 μm long and 22–24 μm wide. 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. Developmentally the secondary thickening meristem is divided functionally into two distinct types: (*i*) the segment of cambium producing conducting elements of sec-

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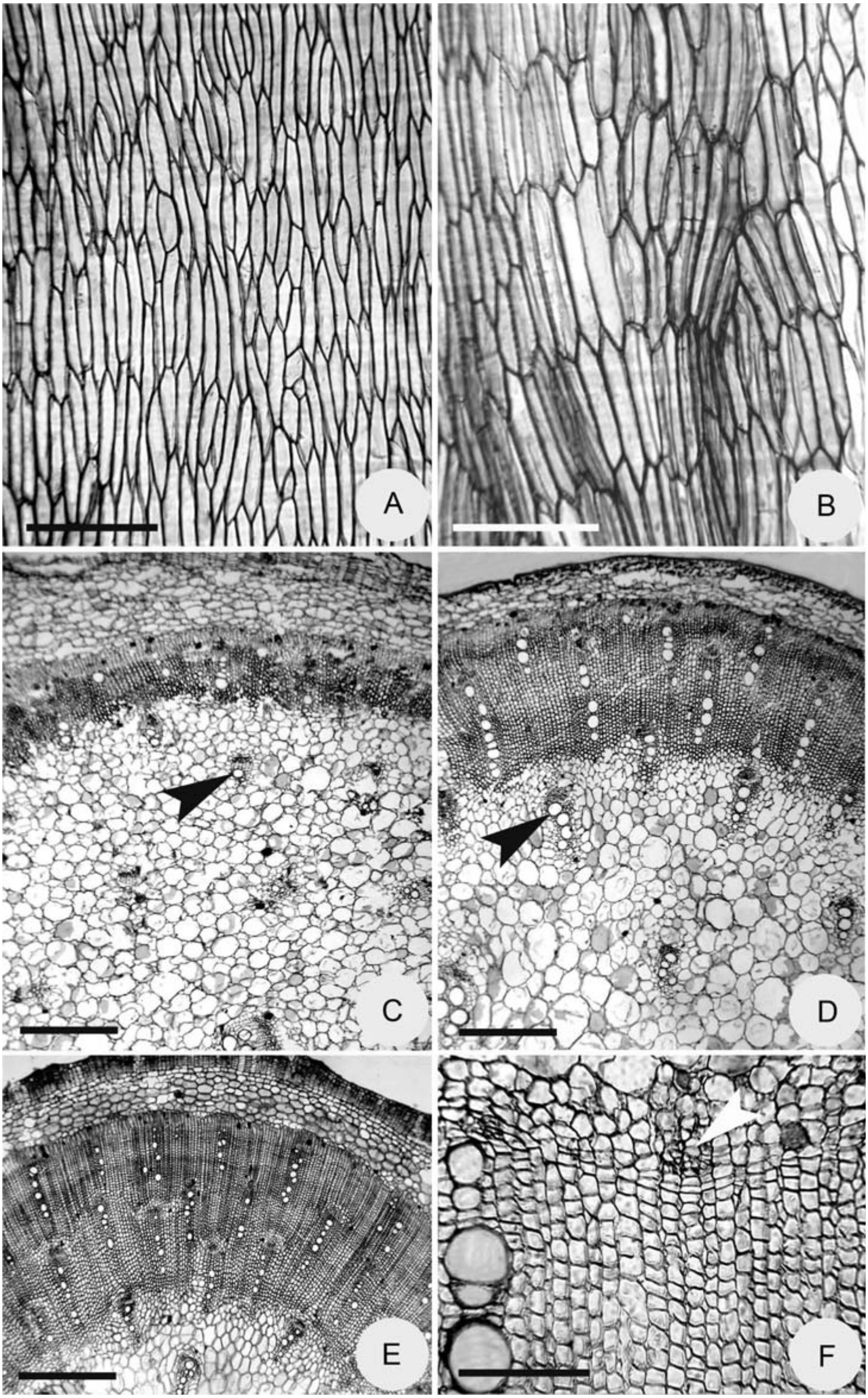
Fig. 1. Tangential longitudinal (A, B) and transverse view (C, F) of cambium and xylem of *Mirabilis jalapa*. A – rayless semi-storied arrangement of cambial cells exclusively composed of fusiform cells. B – enlarged view of cambium showing most of the fusiform cambial cells in storied arrangement. C – young stem with very few xylem derivatives. Arrowhead indicates medullary bundle. D – relatively mature stem showing more secondary xylem and larger medullary bundles (arrowhead). E – normal mature stem showing secondary xylem. F – Master cambium showing only phloem elements forming towards periphery; xylem elements absent on inner side of cambium. Arrowhead indicates sieve tube elements. Scale bar = 100 μm.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ondary xylem and phloem (the vascular cambium of Carlquist 2007) and (*ii*) alternating segments producing only thick-walled conjunctive tissue centripetally and thin-walled conjunctive tissue (parenchyma) centrifugally (master cambium). In the former type (vascular cambium), development of vessels and sieve tube elements remains restricted to a few cambial cells (Fig. 1D, E). On the other hand, master cambium usually produces only conjunctive tissue on either side, but occasionally only sieve tube elements are formed in some places (Fig. 1F).

In young stems, several collateral vascular bundles become connected by interfascicular cambium and form a continuous ring of cambium (Fig. 1C). After the development of 25–30 xylem derivatives, only small segments of cambium cease to divide while alternating segments remain functional. A new segment of cambium develops from the parenchyma cells outside the phloem to replace these nonfunctional segments, and forms a complete ring (Fig. 2A). Cessation of cell division is observed only in segments of vascular cambium that are producing conducting elements of secondary xylem and phloem; continuous activity is observed in the alternating segments that produce only conjunctive tissues on both the xylem and phloem sides. The vascular cambium segments that cease to divide usually maintain a radial arrangement of cambial cells, but occasionally the entire segment differentiates into its derivatives (Fig. 2A, B). In such cambial segments the phloem elements are directly in contact with the xylem derivatives, as in monocots (Fig. 2A).

STRUCTURE AND DEVELOPMENT OF VASCULAR TISSUES

The mature stem is composed exclusively of axial elements, and rays are absent (Fig. 2B, C). The small segments of vascular cambium divide bidirectionally and give rise to conducting elements of xylem and phloem; the alternating segments give rise to conjunctive tissue only. However, the fibres produced by the former type of cambial segment do not differ much from the thick-walled conjunctive tissues produced by the alternating segments (Fig. 2C). The fibres, measuring 580–695 μm in length and 22–30 μm in width, are 2–2.5 times longer than the fusiform cambial cells. Unlike in other dicots, the cambial initials and xylem mother cells do not divide further but differentiate directly into vascular elements, thus maintaining the arrangement in regular radial files (Fig. 2B).

Development of xylem from the vascular cambium precedes that of phloem, and more xylem than phloem cells are differentiated. The ratio of xylem to phloem ranges from 6:1 to 10:2, which makes cambium development appear unidirectional (Fig. 2A). Each phloem mother cell undergoes further divisions, resulting in the development of one sieve element and one parenchyma or two sieve elements. Prior to the development of phloem elements, each newly developed cambial segment produces parenchyma cells externally, followed by sieve tube elements. These parenchyma cells act as a site for the development of a new segment of vascular cambium. Cessation of cell division in the vascular cambium and the continuous activity of lateral meristem encircling the phloem gives rise to the development of phloem within the xylem, appearing as islands in section (Fig. 2A, B).

The rayless xylem is semi-storied and composed exclusively of vessels, fibres and scattered islands of phloem embedded in it (Fig. 2B, C). Vessel elements are mostly solitary and arranged in radial files or in radial multiples of 2–3 vessels. They are mostly angular in transverse view, with a simple perforation plate on their slightly oblique to transverse end walls, with oval to polygonal bordered pits alternating on their lateral walls. Also observed frequently were transitions from elongated pits in scalariform arrangement to short circular pits in the opposite arrangement. The vessel elements, ranging from 140 to 230 μm long, are shorter than the fusiform cambial cells.

MEDULLARY BUNDLES

The vascular cambium present between the xylem and phloem of the medullary bundles maintains its radial alignment even at the senescent stage of the stem and remains functionally active. In mature stems, however, cell division and differentiation of xylem and phloem proceed very slowly, with no significant increase in the size of the medullary

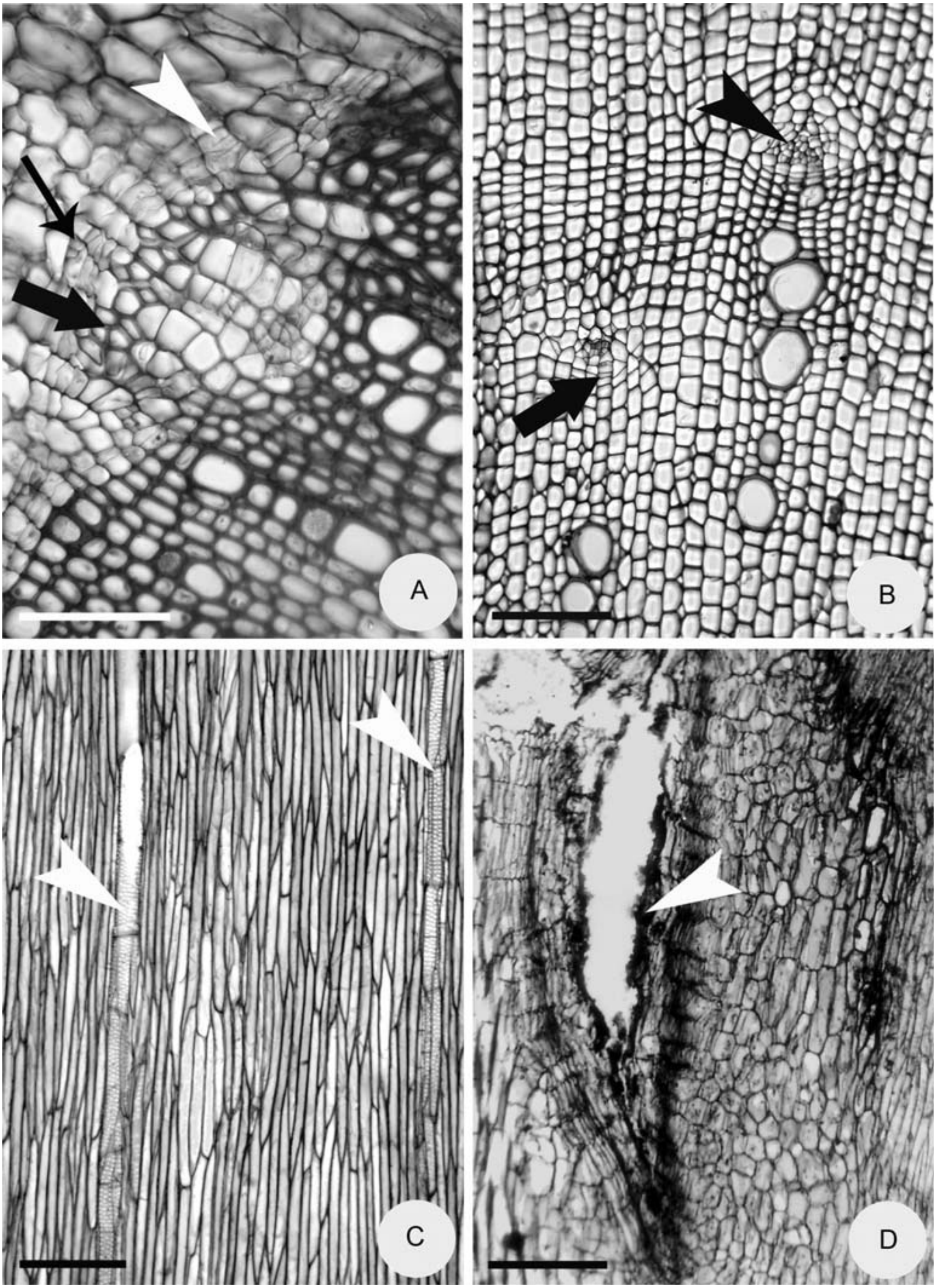

Fig. 2. Tangential longitudinal (A, B) and transverse view (C, D) of cambium and xylem of *Mirabilis jalapa*. A – xylem and phloem differentiating bidirectionally in cambium. Arrow indicates recently developed xylem element and a sieve tube element (small arrow). Note differentiating segment of cambium (arrowhead). B – structure of xylem in mature stem. Xylem fibres show radial alignment. C – rayless xylem showing xylem fibres and vessel elements (arrowheads). Note semi-storied arrangement of xylem fibres and conjunctive tissue. D – experimentally injured portion (arrowhead) of stem. Scale bar = 100 µm.

bundle even at the senescent stage. In young stems the medullary bundles enlarge by the addition of vascular elements next to the cambial segment sandwiched between the xylem and phloem (Fig. 1C–E). The segment of cambium in the medullary bundles is 3–4-layered in transverse section and shows bi-directional differentiation of xylem and phloem. In an entire medullary bundle, vessel wall thickening shows a wide range of variation from helical to alternate bordered pits, with transitional forms between them representing primary to secondary xylem. The vessel elements, 190–215 μm long and 35–65 μm wide, are shorter and narrower than the vessels of secondary xylem. Phloem close to the cambial segment remains functional even at the senescent stage and structurally resembles phloem produced by vascular cambium. The nonfunctional phloem away from the cambial segment is characterised by heavy accumulation of callose followed by obliteration of that phloem; functional phloem possesses open sieve pores even at the senescent stage.

EXPERIMENTAL INDUCTION OF WOUND TISSUE

In injured stems the cambium did not respond to injury as well as it did to patch grafting. Of the ten plants in the grafting experiment, not a single plant showed union of the graft with the stock. Interestingly, no wound meristem/tissue formed in response to any of the different types of injury (pinning, grafting, simple vertical or horizontal cuts, reorientation of patches, etc.). The cambium simply ceased to divide at the injured site and formed a periderm around the injury (Figs. 2D, 3A, B, D). The grafted part gradually dried and turned pale brownish, while production of xylem continued in the adjacent intact portion of the stem (Fig. 3C). The dimensions of vessel elements and xylem fibres remained more or less similar to normal in the xylem produced from the intact cambium between two adjacent injuries.

DISCUSSION

Various anomalous modes of secondary growth are known to occur in dicotyledons, but the unique pattern of secondary thickening characteristic of Centrospermae has attracted the attention of many investigators (Fahn & Zimmerman 1982; Kirchoff & Fahn 1984; Timonin 1988; Philipson 1990; Larson 1994; Rao & Rajput 1998; Rajput & Rao 1998, 1999). There is disagreement about the terminology used for the meristem of families such as Aizoaceae, Chenopodiaceae, Nyctaginaceae and Amaranthaceae. Various researchers have differently interpreted how successive cambia are formed and how various types of cells are produced by them. The products of these cambia are very typical, producing a high amount of parenchymatous cells (conjunctive tissue), the thin-walled cells centrifugally and the thick-walled cells centripetally, while very small segments produce conducting elements of xylem and phloem. This behaviour of cambia in these families has led to the development of a different terminology (viz. primary thickening meristem, desmogen, supernumerary cambia, successive cambia, etc.) for this meristem.

Carlquist (2004, 2007) revisited this problem of terminology by studying eight species from seven genera of Nyctaginaceae and also eleven genera of Aizoaceae showing a wide range of habits and diverse growth forms; that work revealed a wide range of products of secondary activity. On the basis of his study of root and stem anatomy, he reinterpreted the meristem and proposed a new term, ‘master cambium’. In the present investigation we reconfirmed the characters he proposed for master cambium. Our observations of *Mirabilis* are in agreement with his findings, except that some of the segments produced only phloem elements on the outer side, with no xylem derivatives on the inner side. According to Carlquist (2004, 2007), master cambium never produces any conducting elements of secondary xylem and phloem. As shown in Figure 1F, we found that some segments of master cambium produce only conducting elements of phloem centrifugally, and centripetally only conjunctive tissue.

Maheshwari (1930) studied the structure and development of medullary bundles, cambium and its derivatives in *Boerhaavia diffusa* and compared them with *Mirabilis jalapa.* He mentioned that the mode of secondary growth in *Mirabilis*

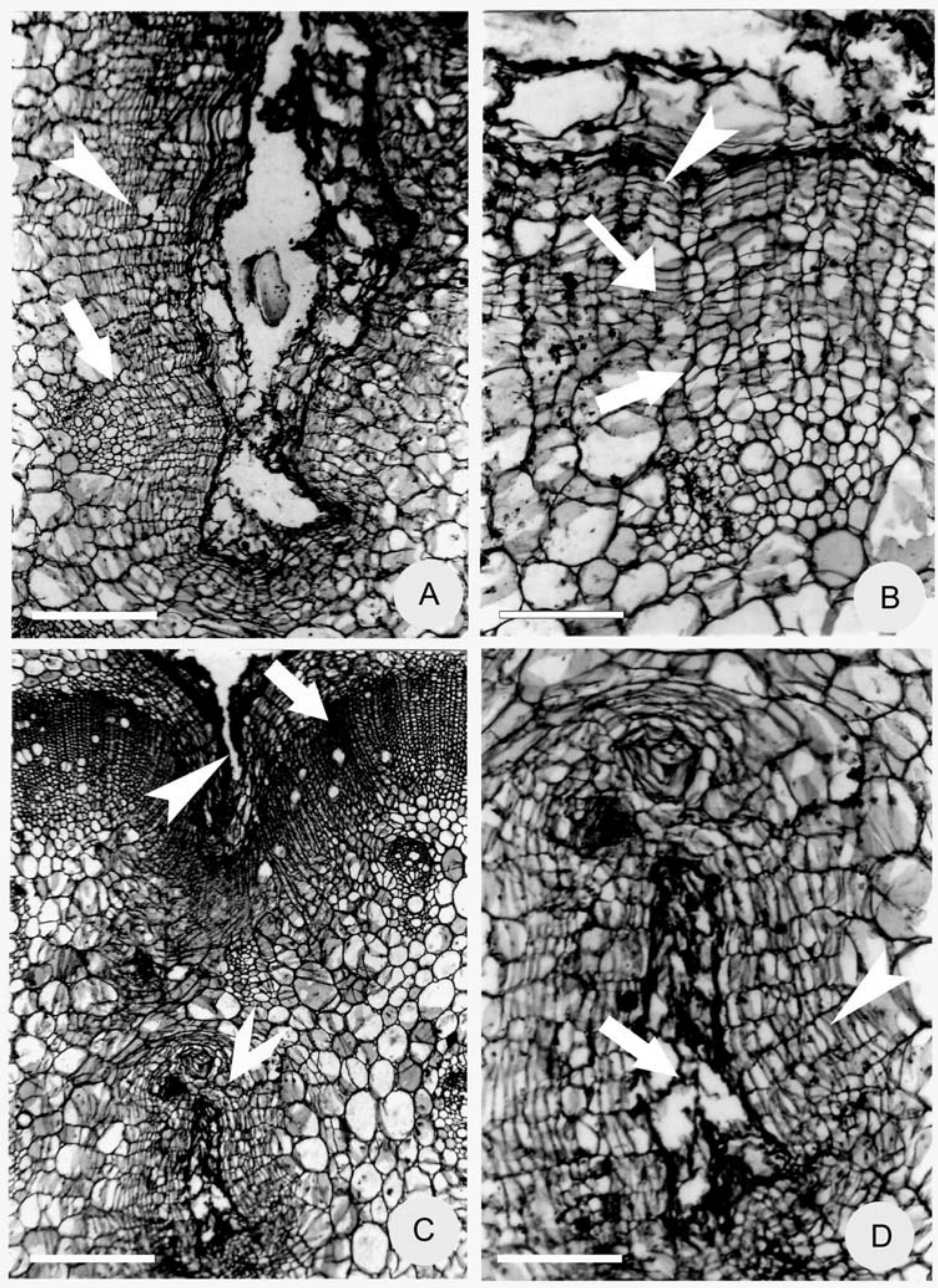

Fig. 3. Transverse view of experimentally injured portion of *Mirabilis jalapa* L. stem. A – formation of periderm in injured stem. Note that cambium ceases to divide after wounding, with no formation of thick-walled xylem elements (arrow). B – enlarged view of stem showing formation of periderm and cambial zone, and cambial zone devoid of xylem and phloem in injured portion. C – no xylem formation after wounding (arrowhead). Note the formation of normal xylem in cambium adjacent to injured portion of cambium (arrow). Arrowhead (lower) shows response of pith cells to injury. D – enlarged view of injured pith cells, showing periclinal divisions followed by differentiation of newly formed cells into parenchyma cells. Scale bar = 100 µm.

is essentially similar to that of *Boerhaavia diffusa*. Our study of mature stem of *Boerhaavia diffusa* showed that after cessation of cell division in one cambium, either a complete new cambial ring develops from the secondary cortex or else tangentially larger segments of new cambium are formed (Rajput & Rao 1998), but in *Mirabilis* tangentially small segments are renewed, giving the appearance of interxylary phloem. In *Borehaavia diffusa*, conducting elements of secondary xylem and phloem are formed from tangentially wider bands, and conjunctive tissues are formed from tangentially relatively narrow bands of lateral meristem, producing successive alternating concentric rings of xylem and phloem.

In *Mirabilis jalapa*, differentiation of xylem and phloem is bidirectional but the rate of cell division towards the phloem side is very slow; development of phloem begins only after the formation of 8–10 xylem derivatives. The number of sieve elements increases slowly following the development of a sufficient amount of phloem prior to cessation of cambial cell division. Their lower ratio as compared to xylem makes the cambium appear functionally unidirectional. Similar features of xylem and phloem development have been reported in *Pupalia lappacea*, a member of Amaranthaceae (Rajput & Rao 1999). However, Balfour (1965) and Philipson and Ward (1965) mentioned that in all members of Amaranthaceae, Chenopodiaceae and Nyctaginaceae the secondary thickening meristem is functionally unidirectional, but its low rate of phloem differentiation as compared to xylem is what makes it appear unidirectional (Rajput & Rao 1999).

The occurrence of rayless xylem has been reported by earlier workers, and it is restricted to a tiny share of dicotyledons (Gibsson 1978; Carlquist 1988, 2004; Lev-Yadun & Aloni 1995; Rao & Rajput 1998, 2003; Rajput & Rao 1999). The absence of rays may be total, with rays not formed at all during the entire life span of the plant, or it may be temporary, with raylessness restricted to the juvenile stage (Lev-Yadun & Aloni 1995). In *Mirabilis jalapa*, rays are absent even at senescence. The occurrence of rayless xylem in dicotyledons is frequently observed in species with reduced cambial activity, short fusiform cambial cells, and highly specialised cell types in secondary xylem. These features are all characteristic of *Mirabilis jalapa*. It has been suggested that rayless xylem tends to occur in plant groups in which normal cambial activity was lost during the course of evolution towards an herbaceous mode of structure (Carlquist 1988). The absence of rays in *Mirabilis* may be associated with its herbaceous habit in which the selective value of radially oriented parenchyma is minimal.

The main purpose of wounding the cambial zone was to induce rays in the *Mirabilis* stem in order to test the hypothesis that wounding induces the formation of more and larger rays. Wounding is known to cause shortening of fusiform initials and transform them into ray initials (Skene 1965; Rier & Shigo 1972; Kuroda & Shimaji 1984; Lev-Yadun & Aloni 1995). It has been shown that decreased axial polar flow of auxin from leaves to roots results in a relative increase of the effect of radial signal flow, which gradually promotes the increase in ray size from the pith outwards (Lev-Yadun & Aloni 1991, 1995). Ethylene disturbs axial auxin flow and increases radial signal flow, thus promoting ray differentiation and decreasing vessel differentiation (Lev-Yadun & Aloni 1995). From the present study it appears that this may be true in plants in which rays already exist but that in *Mirabilis* the cambial zone does not give any response to wounding except for the formation of periderm in the wounded region. Various physiological functions have been ascribed to the formation of interxylary cork. Moss (1940) correlated interxylary cork as a protection mechanism against 'damage from animals, pathogens wind-blown soil', and it is said to help the plants to reduce water loss and restrict upward movement of water through the relatively narrow wound zone. In the experimentally injured *Mirabilis* stems the formation of cork was associated with the wound response to protect the plants from microbial invasion and retard water loss.

ACKNOWLEDGEMENTS. We thank the University Grants Commission (UGC, New Delhi) for financial support, and Dr. Jan J. Wójcicki and the anonymous referee for valuable suggestions on the manuscript.

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Received 13 October 2008