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Structural and developmental studies on cambial variant in *Pupalia lappacea* (Amaranthaceae)

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Secondary growth in the stem of *Pupalia lappacea* (L.) Juss. (Amaranthaceae) resulted in the development of successive rings of cambium. The cambium was storied, consisting only of fusiform cambial cells. Cessation of cell division in each cambial ring was followed by development of a new cambium from the outermost phloem parenchyma produced by the preceding cambium. Absence of ray cambial cells resulted in development of rayless secondary xylem and phloem. Development of conducting elements of xylem and phloem was restricted to the fascicular sector of cambial ring. Interfascicular sector of cambium gave rise to xylem fibres centripetally and axial parenchyma centrifugally. Xylem fibres retained their nucleus even after lignification of cell walls. Raylessness of the stem and possible significance of nucleated fibres are discussed.

Key words: cambium, nucleated fibres, *Pupalia lappacea*, raylessness

INTRODUCTION

Pupalia lappacea (L.) Juss. (Amaranthaceae) is a large straggling undershrub, usually growing along hedges and thorny plants. The structure and development of secondary vascular tissues in the stem of this plant differ from that of the majority of dicotyledons. The stem shows anomalous secondary growth characterised by formation of successive cambial rings. A cambial variant commonly known as anomalous cambium is not uncommon in dicotyledonous plants. There has been a growing interest on cambial variants in different groups of plants (Kirchoff & Fahn 1984, Lotova & Timonin 1985, Timonin 1987, 1988, Philipson 1990, Larson 1994, Rajput & Rao 1998, Rao & Rajput 1998), because of its significance from ecological and evolutionary points of view (Metcalfe & Chalk 1983, Carlquist 1988).

The structure and development of primary and secondary vascular system of leaf, inflorescence and stem of *Pupalia lappacea* were studied by Joshi (1931, 1937). His findings show that cessation of cell divisions in the fascicular segment of cambium is followed by development of new cambium from the outermost parenchyma, while the interfascicular sector of cambium remains active continuously during stem growth. However, our

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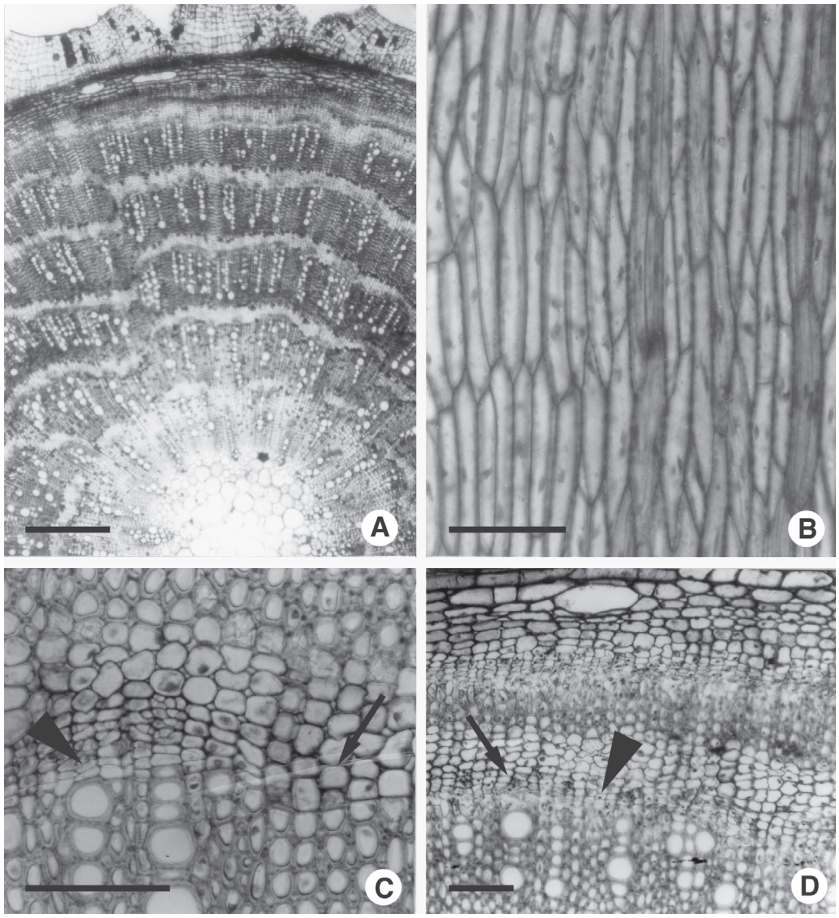

Fig. 1. *Pupalia lappacea.* — A: Stem showing distinct successive rings of secondary xylem. Transverse section. — B: Storied arrangement of fusiform cambial cells. Longitudinal section. Note the absence of ray cambial cells among these cells. — C: Complete differentiation of interfascicular cambial segment (arrow) while the fascicular cambium retains its radial arrangement (arrow head). Transverse section. — D: Development of new cambial ring next to the cortex. Note that fascicular (arrow) and interfascicular (arrow head) regions of previous cambial ring. Transverse section. — Scale bars: 700 μm for A, 100 μm for B–D.

findings of cambial development differ from Joshi's (1931, 1937) results. The present paper, therefore, reports the structure and development of successive cambia and raylessness in the stem of *Pupalia lappacea*.

MATERIALS AND METHODS

Four to eight internodal segments of main stem measuring 3–15 mm in thickness were collected from ten plants of *Pupalia lappacea* growing at Bhorkheda in the northern part of Maharashtra. Samples were immediately fixed in FAA (Berlyn & Miksche 1976) and processed by routine methods to obtain transverse, radial and tangential longitudinal sections 10–15 μm in thickness. For general observations the sections were stained in safranin-fast green (Johansen 1940) for anatomical details and in 4% acetocarmine and I_2KI for localisation of nucleus and starch respectively.

To obtain the mean length and width of xylem fibers and vessel elements, small pieces of stem were macerated with Jeffrey's fluid (Berlyn & Miksche 1976) at 55–60°C for 24–36 hours. The length of fusiform cambial cells was measured directly from the tangential longitudinal sections passing through the cambium. Mean values of the elements were obtained from one hundred random measurements taken for each element using an ocular micrometer scale.

RESULTS

Structure of vascular cambium

The stem is composed of five to six successive rings of cambia (Fig. 1A) comprised entirely of fusiform cambial cells. The cambium is storied with relatively short cells varying 62–167 μm in length (Fig. 1B). In transverse section, the cambium appears two- to three-layered when non dividing and four- to six-layered during the development of xylem and phloem. However, in the older rings, the interfascicular segments of cambium differentiate completely into parenchyma

cells while the fascicular segments maintain its radial arrangement (Fig. 1C).

Development of vascular cambium

Developmentally each cambial ring is divided into two distinct alternative segments; the fascicular segment giving rise to conducting elements of xylem and phloem and interfascicular segment producing only xylem fibres centripetally and parenchyma cells centrifugally. The young stem is composed of 15–16 collateral bundles. These bundles are connected by interfascicular cambium leading to the formation of a cambial ring. In the first ring, the fascicular cambium ceases to divide after 13–16 xylem derivatives are produced. A new cambial strip originates from the outermost parenchyma produced by fascicular cambium. This newly formed fascicular cambium joins with interfascicular cambium and forms a continuous ring. The second ring of cambium follows a development similar to that of the first ring. Interestingly, from the third ring onwards, a complete ring of new cambium develops from the parenchyma cells produced by the previous cambium (Fig. 1D).

Structure and development of vascular tissues

The first elements to differentiate from both sides of the fascicular segment of cambium are parenchyma cells followed by thick-walled lignified xylem elements centripetally and sieve elements centrifugally. However, in the first two cambial rings, only fascicular segments cease to divide and the newly developed cambium joins with interfascicular cambium. This results in the formation of sickle-shaped patches of phloem surrounded by xylem. However, during the development of the third ring of cambium both fascicular and interfascicular segments differentiate simultaneously from the parenchyma cells (Fig. 2A).

In each cambial ring, development of xylem preceeds that of phloem (Fig. 2B). However, development of sieve elements from phloem mother cells decreases. The ratio of xylem to phloem development varies from 6:1 to 8:1. Each phloem mother cell undergoes a periclinal division resulting in formation of one or two sieve elements (Fig. 2C).

Xylem is composed of vessel members, tracheids, axial parenchyma and fibres (Fig. 2D). Vessels are more angular in the innermost two rings but they become gradually oval to circular in the other rings. Vessels are either solitary, tangential or radial multiples of two to three. In the outermost xylem ring, the number of vessels in radial multiples reaches up to 16 (Fig. 2C). They possess alternate bordered pits with a simple perforation plate on the transverse to slightly oblique end walls. The length and width of vessel members vary from 60 to150 μm and 40 to 95 μm respectively. Xylem fibers retain their cytoplasm and nucleus even after development of secondary walls. The nuclei are oval to oblong and fusiform in shape (Fig. 2E), measuring from 8.4 to 12.7 μm in length and 3 to 5 μm in width. Starch commonly accumulates in the fibre lumen. The fibres are nonseptate with simple pits showing a slit-like aperture on their radial walls. The length and width of fibres measure from 635 to 740 μm and 18 to 23 μm respectively.

DISCUSSION

A number of anomalous modes of secondary thickening are known to occur in dicotyledon stems, but mostly only in a few members of a family. In the Amaranthaceae abnormal secondary thickening is a rule (Balfour 1965, Timonin 1987). Opinions differ on the mode of formation of this secondary growth in the different species (Joshi 1937, Metcalfe & Chalk 1950, Lotova & Timonin 1985, Timonin 1987, 1988). Although a cambial variant exists in many species of the same family, it is expressed differently. Joshi (1937) reported that in *Pupalia* new cambium always arises from the parenchyma produced by the previous fascicular cambial segment. To complete the cambial ring, it becomes connected with interfascicular cambial segment, which is continuously active. Our study reveals that this is true only for the first two successive rings of cambia, but from the third ring onwards an entire new ring of cambium consisting of fascicular and interfascicular segments originates from the outermost parenchyma which are produced by the previous cambium.

The outermost phloem parenchyma developed

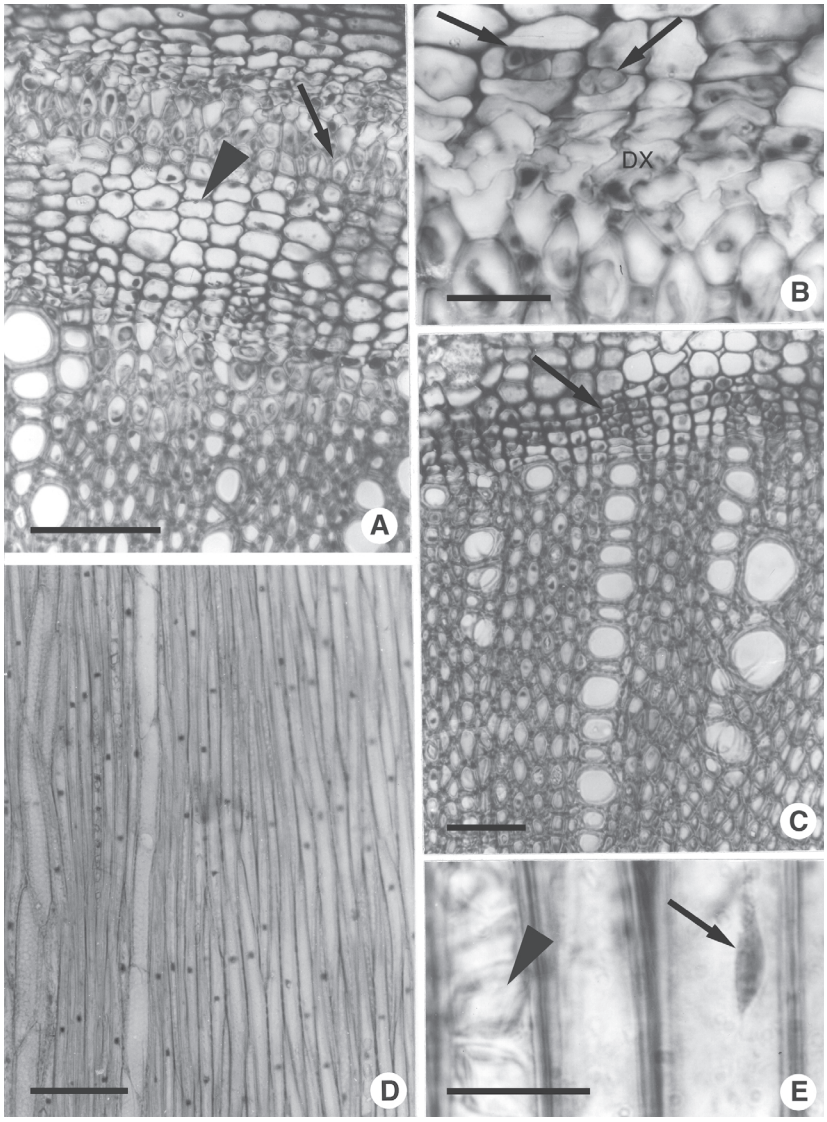

Fig. 2. *Pupalia lappacea*. — A: Enlarged view of stem showing development of lignified xylem elements (arrow) after the formation of parenchyma cells from newly developed cambium (arrow head). Transverse section. — B: Bidirectional differentiation of cambium showing differentiating xylem (DX) and phloem (arrows). Transverse section. — C: Development of vessels and sieve elements (arrow) restricted to only one radial file of cambial cells. Transverse section. — D: Rayless xylem showing xylem fibres and a vessel. Note the absence of rays in the xylem. Longitudinal section. — E: One of the fibres with nucleus (arrow). Arrowhead indicates fibre lumen filled with rhomboidal crystals. Longitudinal section. — Scale bars: 100 μm.

from the preceding cambium gives rise to new cambium in *Pupalia*. A similar mode of cambial development has also been reported in *Boerhaavia* of the Nyctaginaceae (Maheshwari 1930, Rajput & Rao 1998) and in *Trianthema monogyna* L. of the Aizoaceae (Rao & Rajput 1998). In all the members of the Amaranthaceae, in which the stem gains thickness through secondary growth, the growth is considered to be unidirectional (Balfour 1965, Philipson & Ward 1965). In *Pupalia,* the development of sieve elements starts when six to eight xylem derivatives have already been produced. The number of sieve elements increases slowly following the development of sufficient amount of phloem prior to the cessation of cambial cell division. However, their lower ratio as compared to xylem gives an impression of unidirectional development of cambium.

Although cambial variant and xylem structure are known (Joshi 1931, 1937) the raylessness and occurrence of nucleated fibres in the stem have not been reported before for *Pupalia*. The raylessness is predominantly restricted to few dicotyledons and it generally appears in plants having limited cambial activity (Carlquist 1970, Rao & Rajput 1998, Rajput & Rao 1998). It tends to occur

in plants in which woodiness is in the process of increase rather than decrease (Carlquist 1970). It appears that raylessness is mostly confined to the herbaceous species with a limited cambial growth.

Though the absence of rays has been reported in some genera (Barghoorn 1941, Paliwal & Srivastava 1969, Carlquist 1970, Rao & Rajput 1998, Rajput & Rao 1998) the mode of radial conduction in the stem is not yet clear. The occurrence of nucleated fibres in *Pupalia* may be associated with the rayless xylem. The fibres may be functioning both as mechanical and storage elements. On the other hand, the presence of pits on the tangential walls and accumulation of starch in the lumen confirms that the xylem fibres are involved in the radial transport of photosynthates in the absence of rays.

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