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HISTOCHEMISTRY OF CAMBIUM IN *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH. AND *MANGIFERA INDICA* L: ITS SEASONAL CHANGES

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Abstract

Seasonal histochemical localization of starch, lipids, proteins, tannins and crystals in fusiform and ray initials and their derivatives of *Holoptelea integrifolia* and *Mangifera indica* was carried out. The seasonal changes were correlated with the cambial activity of the trees.

Introduction

Even though there are a couple of studies on the histo-chemistry of heartwood formation in tropical trees (Nair *et al.*, 1981; Shah *et al.*, 1981; Ramaiah & Shah, 1985), except a few (Catesson, 1964; Tsuda & Shimaji, 1971; Rao & Dave, 1983), the seasonal histochemical changes occurring in the cambial tissue remain unexplored. Due to its fragility the cambial tissue is seldom used for histochemical studies (Rao & Dave 1983). In continuation to the work on cambial activity of *Mangifera indica* (Dave & Rao, 1982) and *Holoptelea integrifolia* (Rao & Dave, 1985), the present studies were undertaken to elucidate the seasonal histochemical status of the cambial tissue in the two species.

Materials and Methods

Periodic sampling of cambial tissue from *M. indica* L. (Anacardiaceae) and *H. integrifolia* (Roxb.) Planch. (Ulmaceae) was carried out as described earlier (Dave & Rao, 1982; Rao & Dave, 1985). Simultaneously phenological data were also noted. Using conventional procedures for microtomy 10-15 μm thick radial longitudinal sections were stained with Toludine blue (O'Brien *et al.*, 1964) and tannic acid – ferric chloride (Foster, 1934) for general histology, crystals and tannins. Besides, I KI was used for localizing starch (Johansen, 1940), Sudan black for lipids (Jensen, 1962) and mercuric bromophenol blue for proteins (Mazia *et al.*, 1953).

Results

H. integrifolia has storied cambium, whereas in *M. indica* it is nonstoried. Both the cambia have vertically elongated fusiform initials and isodiametric or horizontally elongated ray initials. As described earlier (Dave & Rao, 1982; Rao & Dave, 1985) the cambial activity is continuous in *Mangifera* and varies with well demarcated active and dormant periods of growth in *Holoptelea.*

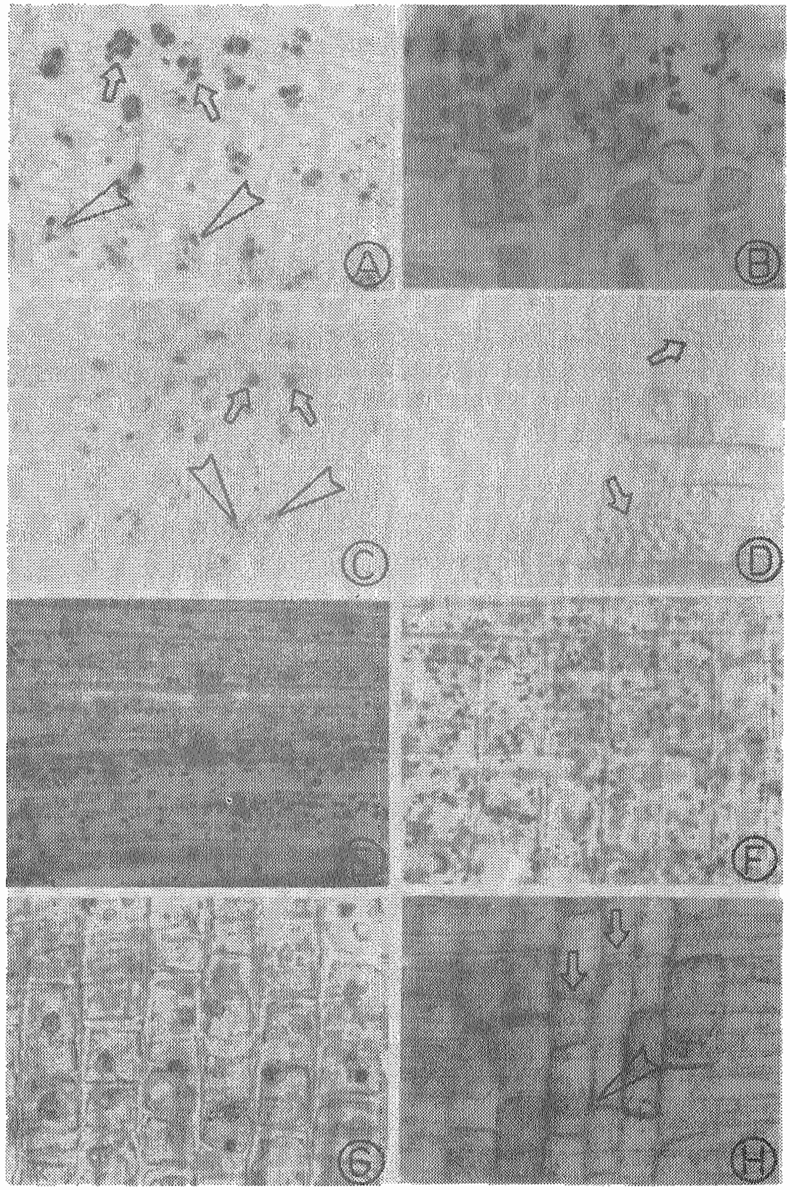

Fig. 1. A-C, E-G. *Holoptelea integrifolia*. Figs. D & H. *Mangifera indica*. All radial longitudinal sections. A-C: Starch in ray initials and its derivatives. A x 580, B x 340, C x 500. D: Unstained section of xylem near cambial zone showing starch grains in Nomarsky interference Microscopy, x 360. G: Lipid bodies in ray initials during active period, x 360. E-F: Lipid bodies in fusiform (E) and ray initials (F) during dormant period. E x 570, F x 400. H: Cambial cells showing lipid bodies, x 530.

Starch : In *Holoptelea* the ray initials contain starch during both the active and dormant periods of cambium. However, the size and distribution of starch differ with the derivative tissues. Darkly stained grouped starch grains are present in ray initials (Fig. 1A) while the vascular elements are rich with large starch grains. Only when the trees shed their leaves in February the starch grains disappear from the ray initials but are found to be rich in the vascular elements (Fig. 1B). During the active period of cambium, a few darkly stained small starch grains appear in ray initials (Fig. 1C) and the vascular derivatives have negligible amount of starch (Fig. 1C). But in October starch grain size and distribution increases and they occur in groups (Fig. 1A), while the differentiating vascular elements have darkly stained small starch grains (Fig. 1A). Starch grain size and distribution increase in November and December in ray initials.

In *Mangifera* starch grains are not present in the cambial cells. Differentiating xylem elements are seen containing reddish brown small starch grains while the mature elements are rich with darkly stained, round to oval, simple starch grains (Fig. 1D). Starch content in the xylem elements decreases considerably in August.

Lipids: In *Holoptelea* lipid bodies are localized in both fusiform and ray initials of cambium. The higher concentration and the larger size of lipid bodies in the dormant cambium and the vascular elements (Figs. 1E, F) decrease considerably during the active period (Fig. 1G). The lipid bodies localized in the fusiform and ray cambial cells and their derivatives of *Mangifera* (Figs. 1H, 2A) do not show much variation in their size and distribution, from January to December. The minute lipid bodies in small amounts occur along the walls in ray and fusiform cambial cells (Fig. 1H at arrows), while the differentiating vascular elements have considerably high concentration of large lipid bodies along their walls and lumen (Fig. 1H, 2A).

Proteins: In both the species the protein bodies are poorly localized in fusiform and ray initials of cambium irrespective of the active and dormant periods. They are present mostly along the cell walls of cambium (Fig. 2B). Differentiating vascular elements have negligible amount of protein bodies.

Tannins: In *Holoptelea* ray initials possess tannin contents in February (Figs. 2C, D). Occasionally the ray initials contain tannins completely filling the cell lumen (Fig. 2E). The vascular ray cells are also rich with tannin. In *Mangifera* tannins are localized in ray initials and their derivatives towards xylem and phloem (Fig. 2F). Phloem parenchyma cells adjacent to cambial zone are seen filled with dark brown tannin contents (fig. 2G).

Crystals: In *Holoptelea* crystals are not detected in the ray initials of cambium, while the phloem parenchyma cells adjacent to the cambial zone during dormant period contain

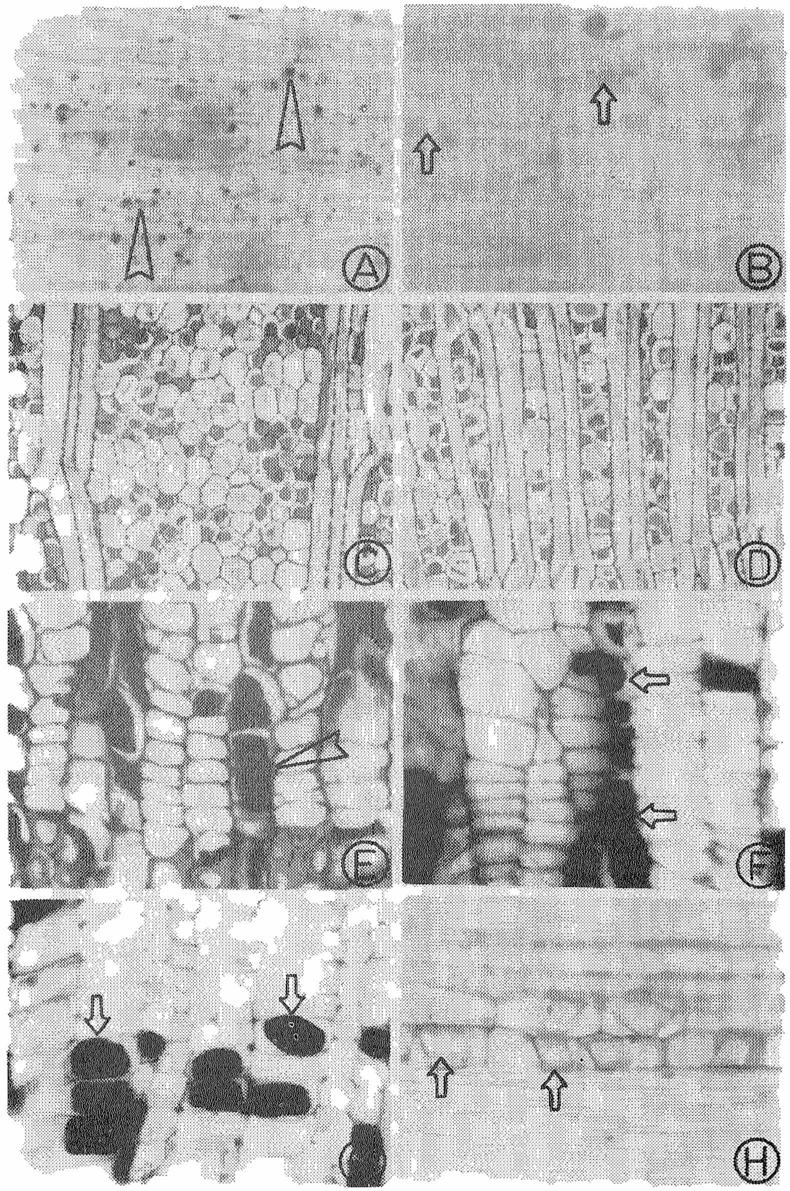

Fig. 2. A, F & G. *Mangifera indica*. Figs. B-E & H. *Holoptelea integrifolia* A, B & H-Radial longitudinal sections, C, D-Tangential longitudinal sections, E-G. Transverse sections. A: Xylem derivatives showing lipid bodies, x 260. B: Protein bodies in fusiform and ray initials, x 500. C: Tannins in ray initials, x 90. D: Tannins in fusiform initials, x 200. E: Tannins completely filling the cell lumen of ray initials, x 620. F: Tannins in ray initials and derivatives, x 530. G: Phloem parenchyma cells filled with tannins, x 320. H: Crystals in the phloem parenchyma cells, x 500.

rhombohedral crystals of calcium oxalate (Fig. 2H). In *Mangifera* also crystal druses occur in xylem and phloem ray cells, which are close to ray initials.

Discussion

The histochemistry of the cambial tissue in *Holoptelea* and *Mangifera* shows some relationship with the cambial activity. Some of the histochemical changes noted in the present study are in accordance with the observations made by Rao & Dave (1983) on *Tectona grandis* and *Gmelina arborea.*

In *Holoptelea* starch is present in both active and dormant periods of cambium. However, in February no starch is localized in ray initials. In *Mangifera* starch is not localized in the cambial cells while the vascular cells are rich in starch. In *Holoptelea* the vascular elements produced during current years cambial activity are without starch while the previous years mature elements are rich in starch. Such similar observation has been made by Rao & Dave (1983). This may be the result of translocation of photosynthates from the developing leaves. In deciduous trees, the building material for shoot development (e.g. starch) comes almost completely from the reserve material (Ziegler, 1964). From the present findings it appears that the starch is utilized for the opening of dormant buds in *Holoptelea*. Busgen & Munch (1927) suggested that a large part of building material for shoot development is supplied by the organs of photosynthesis, the mature leaves in evergreen trees. This may be the reason for the absence of starch in ray initials of *Mangifera* cambium. Recently Ramaiah & Shah (1985) also suggested that the decrease in the amount of starch during June and August in *Lagerstroemia indica* and during July and September in *L. lanceolata* may be due to the high energy requiring processes like flowering and fruiting in the respective periods. Generally, the starch reserves accumulate towards the end of a growing season and are depleted during the cambial activity of the following season (Esau, 1965). In the present study also starch accumulation is noted in the current years production of vascular elements at the end of cambial activity.

Lipid bodies are localized in both fusiform and ray initials of cambium. In *Holoptelea* the lipid body size and distribution which are considerably high during dormant periods of cambium, declines with the reactivation of cambium, resulting in a sparse distribution of minute lipid bodies in the active cambial cells. A similar observation was made by Deshpande (1967) in *Tilia americana* and Rao & Dave (1983) in *Tectona* and *Gmelina.*

In Holoptelea and *Mangifera* cambia, tannins are present in the ray initials. Stewart (1960) noted minimal amount of tannins in the cambial zone of *Eucalyptus regnans* and their increase on each side of it. He suggested that the tannins are waste materials which are excreted to either side of the cambium during metabolism. A recent ultrastructural investigation by Rao (1985) has revealed the tannin accumulation in resting cambium and its subsequent hydrolysis during the onset of spring in *Aesculus hippocastanum*. It appears from the present findings that the presence of tannin in cambial cells may vary from species to species.

Crystals are considered as inorganic waste products formed as a result of metabolic processes of the cells. In the present investigation druses of calcium oxalate crystals have been observed in the ray initial derivatives towards phloem in *Mangifera*. Rao & Dave (1984) have reported the occurrence of crystal in the ray initials (druses in *Tectona* and sheaf of long accicular raphides in *Gmelina*). In *Holoptelea* crystals are absent in ray initials. On the contrary, the phloem parenchyma cells adjacent to the cambial zone contain crystals. According to Rao & Dave (1984) the appearance of crystals in ray initials is puzzling, when the fact is considered that during active divisions meristematic cells mostly lack ergastic inclusions (Esau, 1965). But according to Chattaway (1953) since the crystals may first be observed directly behind the cambium, they may be metabolic by-products of physiologically active cells.

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