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DEVELOPMENT OF VASCULAR CAMBIUM IN THE LEAF RACHIS OF *KIGELIA AFRICANA* (LAM.) BENTH

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ABSTRACT

The structural changes during the vascular cambium development from procambium in the leaf rachis of *Kigelia africana* bearing leaflets of different developmental stages (young, mature and old rachis) were investigated. The different regions from the internodes of rachis bearing young, copper brown coloured leaves revealed the sequential stages of cambial development. Terminal region of the rachis showed developing procambium along with protoxylem and protopholem derivatives, middle regions exhibited the development of interfascicular cambium whereas basal region showed complete ring of vascular cambium with secondary phloem derivatives. Rachis bearing brownish coloured leaflets showed an active cambium with both secondary xylem and phloem derivatives indicating bidirectional cambial growth. Rachis with fully matured dark green leaflets was characterized by the presence of dormant cambium surrounded by mature xylem and phloem elements and occurrence of calcium oxalate crystals in the phloem parenchyma cells. The study also describes the pattern of vascular cambial development in the leaf rachis of *Kigelia*, a deciduous tree and it was compared with development of vascular cambium in the leaves of conifers and evergreen dicotyledons. Influence of leaf maturity on cambial activity and secondary xylem development is discussed.

Key words: *Vascular cambium development, leaf rachis, Kigelia africana, pattern of secondary growth*

INTRODUCTION

In all plants, efficient delivery of water dissolved nutrients and transfer of fixed carbon are vital for plant survival. Plants solve this problem through the development of an integrated network of veins (the vascular system) that interconnect all parts of the plant. These vascular tissues are differentiated from the meristematic cells: - i.e. procambial cells during primary growth and the vascular cambium during secondary growth. Procambium is the primary vascular meristem that is segregated from the shoot apical meristem to become the leaf traces or vascular bundles that unites leaves with stem during elongation or primary phase of growth. Leaf vein design is fast expressed as a pattern of procambial strands that extends from slim vascular bundle into leaf parenchyma to form primary vein followed by basipetal areas of secondary vein as continuous loops [3]. Procambial cells are characterized by their elongated shape, lack of vacuolation and formation of narrow files [10, 15, 17). Although structural aspects and origin of procambium followed by sequential development of vascular cambium in shoots and roots of many plants have been studied in detail by earlier workers (8, 9, 10, 18), meagre information is available on the pattern of cambial development in lateral organ like leaves. Tremendous growth of trees is directly related to their extensive canopy, which forms the nutritional source for their growth and development. Thus, it is important to study the development of vascular tissues in leaves. Considering the complexity of identifying cambial initials, it is necessary to study the leaves thoroughly in the process of ontogenesis, with constant comparison of separate stages of the vascular system development. Therefore, it is envisaged that the present study would elucidate the sequential stages in the development of vascular cambium.

MATERIALS AND METHODS

Actively growing and mature current year's leaves were collected from *Kigelia africana*, growing in the premises of B R Doshi School of Biosciences, Sardar Patel University, Vallabh Vidyanagar (22°34' N, 72° 56'E). The intermodal regions of rachis were categorized into three developmental stages based on the age of the leaflets that is indicated by their colour; young with copper brown coloured leaflets, immatureness with brownish green leaflets and old rachis bearing dark green coloured leaflets. Terminal, middle and basal part of the leaf rachis from different internodes of growing shoot were collected to study the development of the vascular cambium. Samples were fixed in FAA (Formaldehyde- Acetic Acid- Alcohol) immediately after removal from the growing shoots. These samples were dehydrated in Tertiary Butyl Alcohol (TBA) series and embedded in paraffin wax [2]. Transverse sections of 10 to 12 µm thick were cut by rotary microtome (Zeus) and stained with Toluidine Blue (prepared in Benzoate buffer, *p*H 4.2) for the general histological studies [5]. After passing through ethanol xylene series sections were mounted in DPX. Stained sections were observed and important results were photomicrographed by using Nikon Microscope attached with R CCD-3 camera.

RESULTS AND DISCUSSION

In majority of trees defoliation starts in January, and continues till the first week of February. Defoliation is concomitantly followed by swelling of the terminal bud is observed in one year old shoots. Sprouting of young shoots along with brown coloured leaflets continues till the end of February. Shoot produced in the beginning develops brown coloured leaves which later turn into green after completion of shot growth.

Terminal, middle and basal part of the leaf rachis of brown colored leaves obtained from actively growing of shoots revealed different stages of cambial development. During the sequential transition from procambium to vascular cambium, primary xylem and primary phloem occur first at the tip of elongating leaf rachis while secondary xylem and phloem occurs later towards the base gradually. Similar basipetal pattern of vascular development has been reported in the needle of *Pinus strobilus* (12). Vascular bundle with distinct fascicular cambium and its early derivatives were observed in

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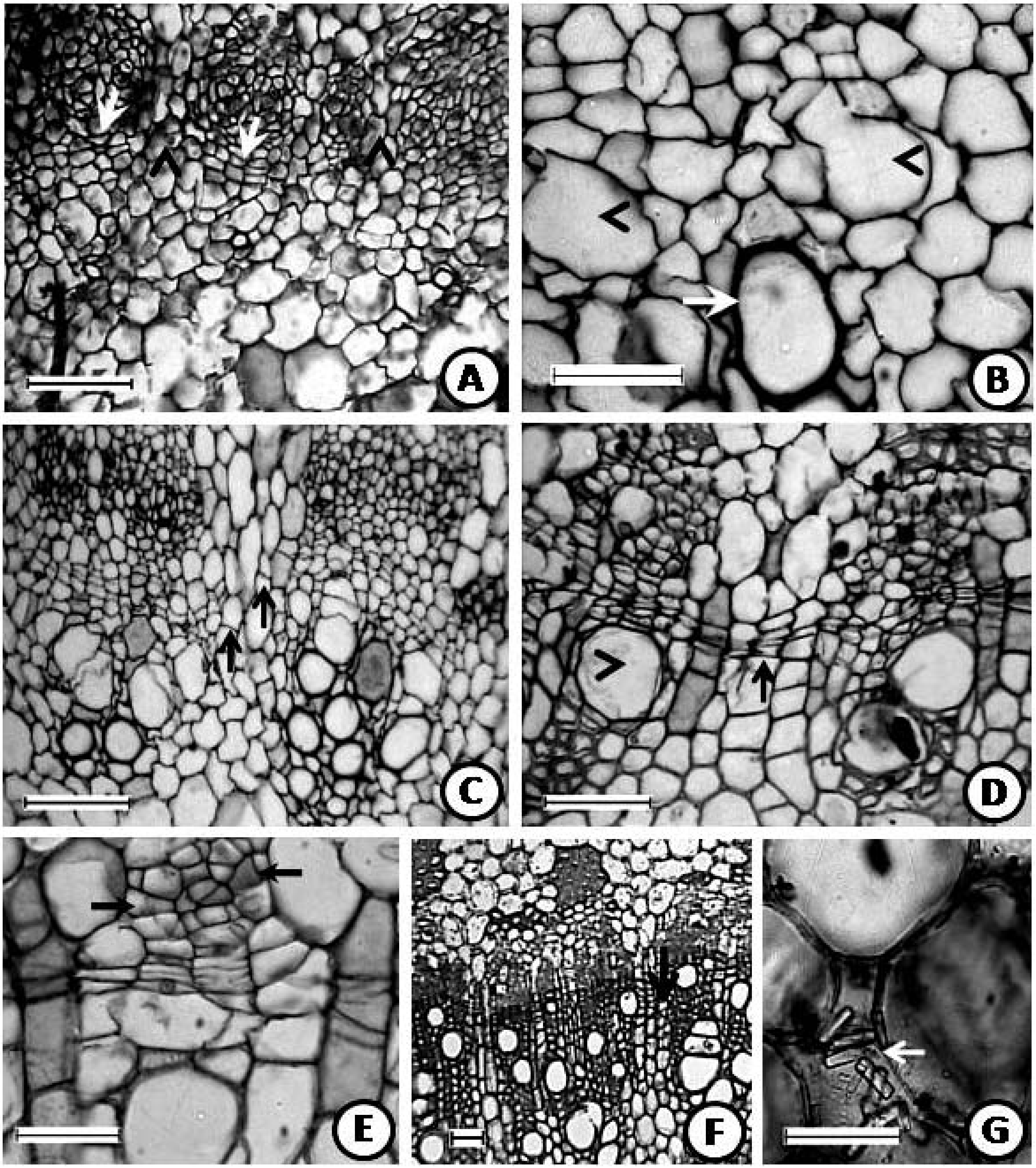

FIGURE: Transectional views of the leaf rachis showing cambial development.

A. Terminal part of the leaf rachis showing interfascicular parenchyma cells (arrow head) between adjacent vascular bundles. Primary vascular bundles with the new fascicular cambium (arrows). (Bar=100 µm).

B. The terminal part of leaf rachis with copper brown color leaflets showing the tracheary element formed from fascicular cambium (arrow) from. Note the newly differentiating tracheary elements (arrowhead) (Bar=50 µm).

C. The middle part of the leaf rachis showing development of inter fascicular cambial cells (arrows) by tangential divisions of interfascicular parenchyma cells (Bar=100 µm).

D. Basal part of leaf rachis showing complete differentiation of interfascicular cambial segment (arrow) which is joined with fascicular cambium the base of leaf rachis (Bar=100 µm). Arrow head shows the differentiated metaxylem tracheary element.

E. Base of leaf rachis showing newly developed primary phloem elements (arrow) from the cambium (Bar=50 µm). Leaf rachis of green colored leaflets showing a ring of vascular cambium (arrow) with differentiating xylem & phloem. (Bar= 200µm).

the terminal part of the leaf rachis. The fascicular cambium was composed of 2-3 layers of isodiametric cells. Procambium consists of cells with smaller elements, which appear first in the periphery of central ground meristems consisting of two to three procambial cells in the fascicular region of the cambium [18]. Vascular bundles in the terminal part of the rachis were separated by radially elongated interfascicular parenchyma cells (Fig.A). The first formed protoxylem element was followed by differentiation of metaxylem trachery elements (Fig.B). Differentiation of protoxylem from procambium of stem is usually endarch or centrifugal [7]. In the middle part of leaf rachis, the interfascicular parenchyma cells underwent repeated periclinal divisions leading to the development of a layer of interfascicular cambium (Fig.C). Protoxylem differentiation was followed by development of metaxylem elements. Maturation of metaxylem elements in the vascular bundle was evident from their thick lignified secondary walls with larger diameter as compared to protoxylem elements. At this stage, meristamatic in nature and underwent periclinal divisions leading to the development of interfascicular cambium [8].

In the basal part of the rachis the fascicular cambium joined with the interfascicular cambium and gave rise to the vascular cambium and thick walled metaxylem elements (Fig.D). At this stage, protopholem bundles appeared more distinct with well- developed sieve elements close to the newly formed cambial ring (Fig.E). However, no secondary growth was observed in the basal part of rachis bearing brown colored leaflets. In majority of angiosperms, the protoxylem differentiation occurs acropetally into the leaf primodia and is continuous with existing phloem in the axis [9]. The protoxylem on the other hand, usually differentiates first at the base of the leaf primodium at its junction with the axis and basipetally into stem, and therefore, xylem is initially discontinuous [7]. The continuous structure of phloem elements during initial stage itself might be related to translocation of nutrients and other substances to procambium or parenchyma to support their meristamatic activity in *Kigelia*. Though basal part of the rachis bearing brown colored leaflets rachis did not exhibit prominent secondary growth, the same region of rachis became brownish green leaflets showed a ring of xylem with vessel, fibres and parenchyma cells (Fig. 1F). This shows that physiological maturity of vascular cambium is closely associated with maturity of leaves. Cambial activity is far easier to make for the xylem elements than for the phloem elements, because of definite structure of the tracheary elements, their relative longevity and of the considerable volume which is occupied by the xylem (21). Production of xylem in the coniferous needle is limited by an unidentified trachied differentiation factor (19, 20). Ewers and Aloni suggested that this factor is produced in young leaves but not in older leaves and therefore xylem production is limited in the former (12). Cambial cell division and xylem differentiation have been closely related to phytohormonal signals [1, 4, 14]. Possibly, the brownish green colored leaflets provide more auxin which is reported to boost xylem differentiation [13]. On the other hand, cambium in the rachis bearing fully matured green leaflets was dormant with 2-3 layers of thick walled cambial cells. The reports on secondary growth pattern in the leaves of evergreen dicotyledons are controversial. Elliot (6) reported well differentiated secondary xylem with distinct growth rings while there was no secondary phloem in some species. On the other hand, Frank (13) reported both, secondary xylem and secondary phloem development in the leaves of *Ilex aquifolium*. Based on the study of cambial activity in the leaves of five species each of deciduous and evergreen trees, Shtromberg (21) suggested that the cambium is either altogether absent or does not show any noticeable activity in the leaves of deciduous trees while evergreen species showed considerable cambial activity. On the contrary, the present studies shows that the secondary growth producing a considerable amount of secondary xylem and phloem after leaf maturation. This growth pattern observed in rachis is similar to the pattern in shoots.

Cambia initials in the mature leaves of *Pinus* produced secondary phloem but no xylem production was observed indicating unidirectional vascular cambium in the first growing season, while bidirectional growth begins with the next season (11). In *Kigelia*, although copper brown leaflets showed more phloem production and no secondary xylem immediately after the development of vascular cambium, the rachis bearing mature, brownish green coloured leaflets showed secondary xylem production. In *Pinus* leaves, the transition from uni-to-bidirectional cambial growth phase is about one year (11) whereas in *Kigelia*, this transition period is about one week. Therefore, we hypothesize that a common pattern of vascular development exists in both conifers and angiosperms particularly in dicots. However, the transition from uni-to-bi directional cambial growth would be faster in the leaves of dicots as compared to conifers.

Phloem parenchyma cells near the dormant cambium of rachis bearing dark green older leaves showed the presence of needle shaped crystals of calcium oxalate (Fig. G). Crystals formation is a mechanism to regulate calcium level in plants organs [16]. Crystals in plants may function as a means of removing the calcium oxalate which may otherwise accumulate in toxic quantities. In addition, it has been suggested that the crystals serve purely as structural supports or as a protective device against foraging animals.

In conclusion, the present study reveals the patterns of vascular cambial development from procambium in the leaf rachis of *Kigelia africana*. The development of cambium and xylem differentiation found to be directly related to the leaf maturity. The developmental aspects show many similarities with that of conifers especially in their uni- to-bidirectional growth transition. However, the confirmation of this view needs further detailed investigation on comparison of pattern of vascular differentiation in the leaves of more dicotyledonous tree species.

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