Pericyclic origin of Monocot meristem – Evidences from *Curcuma* and *Zingiber*

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Abstract

Secondary thickening in Monocots, mostly palms and rhizomatous species, is considered due to a Secondary thickening meristem which is now named as Monocot cambium which originates from pericycle and or endodermis. The present study on four species of Curcuma, *i.e. C. caesia, C. aromatica, C. amada* and *C. longa* as well as *Zingiber officinale* proves conclusively that the monocot cambium is exclusively pericyclic in origin. The cursory term "abnormal secondary growth" given to monocots should be avoided as the secondary growth is always from pericycle and from evidences from molecular data on 80% overlapping of the regulatory principles of cambium regulation between Dicots and Monocots. The present study proves that Endodermis is better considered as the outer membrane of stele, than the innermost membrane of cortex. The prevalence of amphicribral vascular bundles as a primitive character retained in monocots also is suggested.

Key Words: Monocot meristem, Secondary growth, Pericycle, Endodermis, Amphicribral bundle

Introduction

Though all monocotyledons lack a vascular cambium, some of these plants get their stem increased in girth by means of a different type of lateral meristem which is named as a secondary thickening meristem (STM). Lesser commercial significance of monocots is cited as the reason of very limited studies on the secondary thickening of monocots. Secondary vascular system has been recognized within 22 genera belonging to the Asparagales (Rudall, 1995), many palms and rhizomatous species of Zingiberales, Cyperaceae, Bromeliaceae and Commelinaceae. Meristems responsible for secondary growth in monocots are named variously by different workers as "thickening ring " " Etagen cambium", " meristematic zone",

" secondary thickening meristem", " anomalous cambium", "vascular cambium", " accessory cambium", " cambium-like zone", "monocot cambium", "Etagen meristem", " storied meristem"and " storied phellogen" (Joanna *et al,* 2015).

Many authors especially Rudall (1991), suggested a primary thickening meristem (PTM), responsible for the primary thickening of the stem in virtually all monocotyledons. A number of other workers like Cheadle (1937), DeMason (1983), Stevenson and Fisher (1980), DeMason and Wilson (1985) and Gifford and Bayer (1995) attribute PTM the function of promoting the formation of adventitious roots in Monocots.

In a pioneering work, de Menezes and co-workers (2005) observed endodermis with meristematic activity in the root of all the species they have studied *i.e*. in the stem of *Cyperus, Cephalostemum* and *Lagenocarpus rigidus*, and in the leaf trace of *Cyperus* and leaf of *Echinodorus*. Considering the continuity of tissues through the root, stem and leaf, the authors concluded that in monocot stems, the pericycle remains active throughout the life of the plant as the generator of the vascular tissue. The "Primary Thickening Meristem", of earlier workers, is in fact the pericycle plus the endodermis and its derivatives (or only the pericycle). Close to the stem apex, the assemblage of seems to be a unique meristem, giving rise to the inner cortex and vascular tissues.

During our pharmacognostic studies on *Curcuma caesia* Roxb, we observed that the secondary thickening in the rhizome was due to a clear percyclic meristem. As we are interested in this topic we screened other three species of *Curcuma* i.e. *Curcuma amada* Roxb, *C. aromatica* Salisb and *C.longa* Linn. and *Zingiber officinale* to observe their secondary thickening.

Materials and Methods

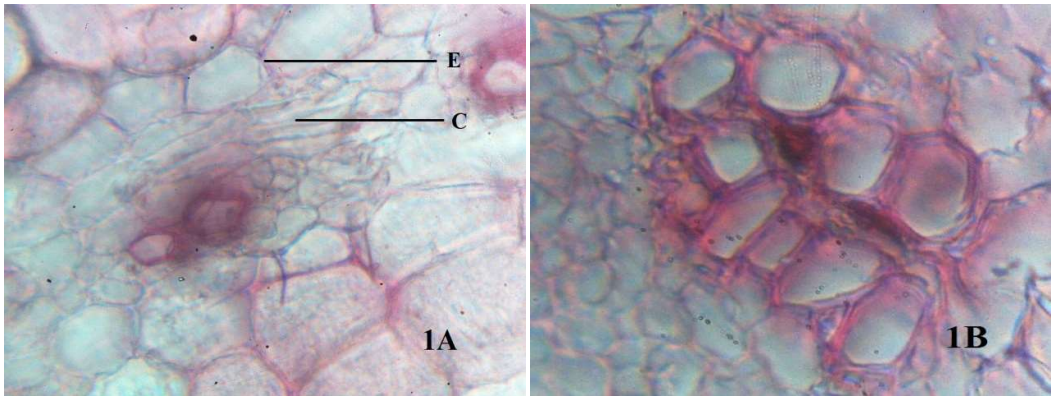
All the plants were collected from the first author's home garden and voucher specimens are deposited in Herbarium of Navrachana University

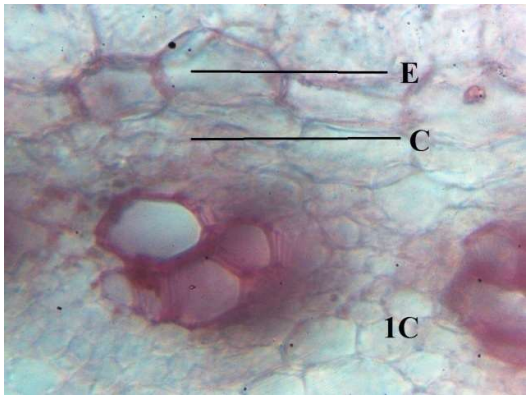
Fresh plant materials were used for initial pharmacognostic studies which involved hand sections. Dry materials were boiled in water to make them soft. Later they were fixed in FAA (Johansen, 1940). Sections were taken and stained with safranin and after dehydration mounted in DPX. The sections were then observed under microscope and photographs were taken with CMOS 5.2 megapixel Microscope camera with basic software. The size (dimensions) of various cells and crystals were measured using stage and ocular micrometers. The quantitative data are based on the average of 20 readings.

Results

Curcuma caesia Roxb

The rhizome of *C.caesia* is divided to two regions, an outer one and a central one, both containing vascular bundles which are mostly collateral and closed. The central region is demarcated by a distinct layer containing an outer endodermis, a meristematic zone below it (derived from pericycle and a ring of both small nascent and large mature vertical and tangentially running vascular bundles). This region appeared as a mechanical wall separating the central region from the outer cortical region. Endodermis was clear as a single layer of barrel shaped cells (25-32mμ in breadth and 12 mμ in height) with casparian thickenings (Fig 1 A). Endodermal cells are clearly distinguishable from the adjoining cortical parenchyma cells which are large isodiametric, stained pink (about 45-50 mμ in diameter) containing large starch grains. Inner to endodermis is seen thin walled cambial cells presumably derived from a pericycle. These cambial cells are rectangular, closely packed without intercellular spaces and in tiers. Inside are the newly forming vascular components, the tracheids and phloem elements, sometimes tangentially arranged. Most of the vascular bundles are collateral but some are amphicribral in nature in that phloem is seen surrounding the xylem (Fig.1B). The ground tissue in centre consisted of isodiametric parenchyma cells similar to that of cortex, containing many elongate starch grains and oil ducts. The outer cortical region also contained isodiametric parenchyma and a number of collateral vascular bundles. Cortex is bordered on the outside by cork cambium of rectangular closely packed cells and mature cork cells.

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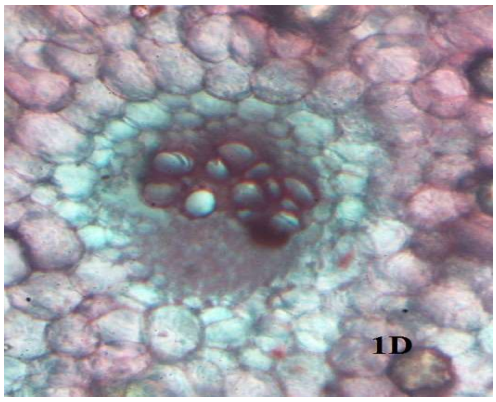
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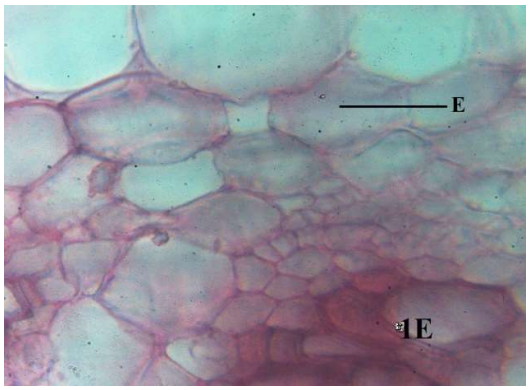
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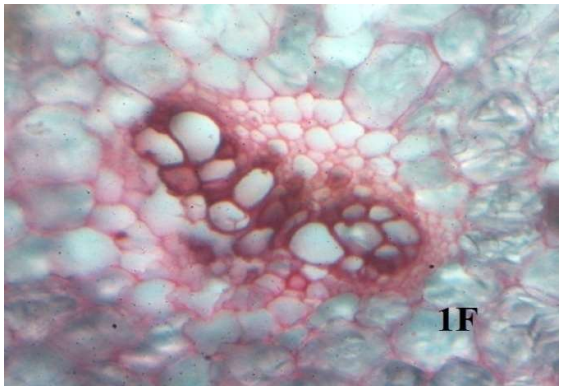
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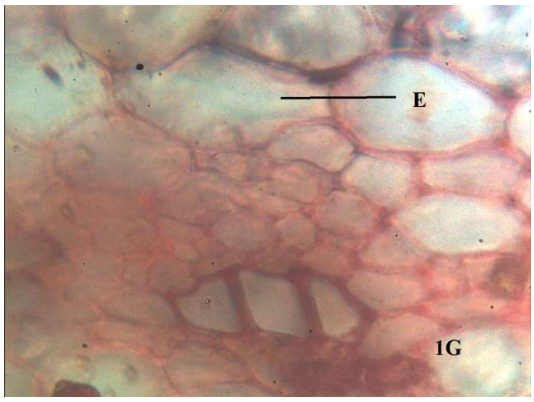
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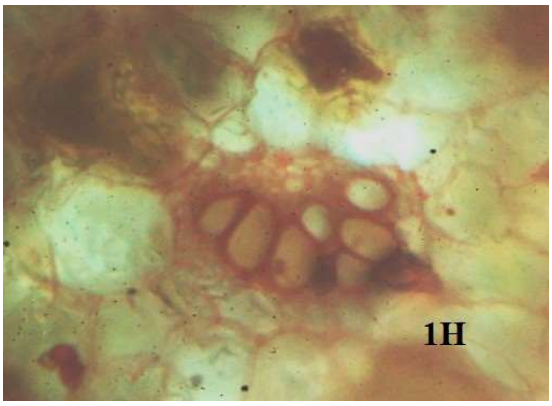
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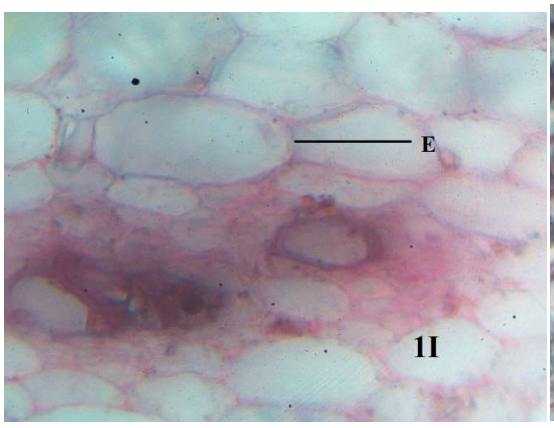
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Fig 1 , A&B, *Curcuma caesia,* A. Vascular bundle of showing endodermis (E) and cambium(C), B. Amphicribal vascular bundle; C& D, *C. Amada*- C.Pericyclic cambium and D. Amphicribral v.b.; E& F, *C. aromatica*- E. Endodermis and F. vascular bundle; G&H. *C.longa*- G. Endodermis and H. Amphicribral vascular bundle; I & J, Zingiber officinale. I. Endodermis and J. amphicribral vascular bundle.

***Curcuma amada* Roxb.**

The central cylinder occupies about 32% of the rhizome with a clear cut endodermis and adjoining vascular ring inside. Cells of endodermis are bigger in size having mostly rectangular (60-65 mμ in breadth and 30 mμ in height) and square shape (30 mμ on one side) barrel shaped cells. (Fig 1C). Cambial cells are narrow and the phloem tissue adjoining are clearly visible. Here most of the bundles are collateral but some are amphicribral in nature in that phloem is seen surrounding the xylem(Fig 1D). Cells of endodermis are clearly distinguishable by their small size and absence of any cytoplasmic inclusions like starch when compared with the large isodiametric cells of cortex (diam.80 mμ) and inner ground tissue (93 mμ diam.)

***Curcuma aromatica* Salisb.**

The central cylinder occupies about 32% of the rhizome with a clear cut endodermis and adjoining vascular ring separating the two regions. Cells of endodermis were short rectangled barrel shaped cells of about 60 mμ in breadth and 40 mμ in height (Fig 1E). Cambial cells and the phloem tissue adjoining are clearly visible. Here the vascular bundles are amphicribal in nature in that phloem is seen surrounding the xylem(Fig. 1F). Cells of endodermis are clearly distinguishable by their small size and absence of any cytoplasmic inclusions like starch when compared with the large isodiametric cells of cortex (diam.80 mμ) and inner ground tissue (93 mμ diam.) .

***Curcuma longa* Linn.**

The differentiation of an outer and inner region is observed in *C. longa* rhizome also. The inner region amounts 44% of the rhizome. Endodermis is visible as a colour less layer and the cells are barrel shaped with a breadth of 50-55 mμ and a height of 37 mμ (Fig 1G). Pericyclic cambial cells are smaller and not in tiers as in other plants. Vascular bundles are amphicribral (Fig 1H) though some appear collateral and closed. Here also, there are tangentially running vascular bundles. Pith cells are very large isodiametric having a diameter of 150 mμ, while cortex consists of smaller isodiametric parenchyma of 90 mμ. Cork cambium is of narrow rectangular cells having half the thickness of cork cells.

***Zingiber officinale* Roscoe**

Of the two regions demarcated by the ring of vascular bundles, the outer region (Cortex with its inclusions) formed a large share of 79%. Endodermal cells are short barrel shaped with 54 mμ breadth and 24 mμ height (Fig. 1I). They are also free of any cell inclusions. Pericyclic cambium is clearly seen at certain places. Similar to *Curcuma aromatica* here also the phloem is seen surrounding the xylem (v.b. amphicribal, Fig 1J). There is a hemispherical covering of sclerenchyma surrounding ¾ th of vascular bundles. Both cortical and ground tissue are having similar size and shape, isodiametric parenchyma having a diameter of 90-95 mμ. Earlier de Menezes and co-workers (2005) also observed that the stem endodermis did not exhibit meristematic activity, and the pericycle, adjacent to the endodermis with Casparian strips, is clearly the generating layer for vascular tissues.

Discussion

The observations on all the four *Curcuma* spp, *i.e. C.caesia*, *C,amada,* C. *aromatica* and *C. longa* and *Zingiber officinale* prove that **the cambium responsible for secondary thickening is pericyclic in origin.** The endodermal layers in all five plants are intact and clearly visible and there are only few anticlinal divisions which help in increasing the girth of stele and there is absolutely no periclinal division needed for the formation of cambium. These cells are entirely different from the bordering innermost cortical cells in shape (barrel shaped), in possessing much smaller size and absence of intercellular spaces. Endodermal layers appear as a clear distinguished layers free of any cellular inclusions like starch, oil or plastids. The cortical cells are much larger (may be 3 to 6 times bigger), isodiametric and filled with starch grains or other inclusions and with intercellular spaces. These results are fully supporting the theory proposed by de Menezes *et al* (2005) that the "Primary Thickening Meristem" which earlier workers named, is in fact originated from pericycle and the secondary thickening is due to pericyclic cambium. Our evidences presented here do not support an endodermal origin of cambium.

Moreover, the secondary growth seen in monocots need not be considered "abnormal secondary growth" (as considered by many anatomists) and should be considered as "true secondary growth', as proposed by Fisher (1973) and DeMason (1994,) because similar to the origin of interfascicular cambium from interfascicular ground tissue inner to pericycle in normal secondary growth in Dicotyledons, here it is the pericycle itself giving rise to secondary cambium producing both secondary vascular tissues and parenchyma in a unidirectional manner. Of late, a comparison between the transcriptomes of the monocot cambium (of *Cordyline australis* and *Yucca gloriosa*) with the Dicot cambium (from *Populus trichocarpa* and *Eucalyptus grandis*) revealed a considerable overlap of more than 80% with 5, 345 of a total of 6, 527 Gene ontology (GO) terms shared among all four species that included key cambial regulators

as *KNAT* class I and class III homeodomain-leucine zipper (HD-Zip III) transcription factors. Only 127 GO terms were uniquely associated with the two monocot species which were not found in dicots. This suggests that in monocots the regulatory principles of cambium regulation may have been reactivated and thus the monocot cambium evolved (Mathew *et al.* 2017).

Another point in which we differ from others is that we prefer to adhere the conventional view that the **endodermis is the outer most layer of stele (Vascular Cylinder) and not part of cortex** as proposed by Van Fleet (1961). The term "Endodermis" indicates that it is a "dermis" meaning a skin that is the outer layer of stele. From our sections of stems of five Zingiberaceae members and from the many sections of roots available outside, endodermis is seen similar to the pericycle and ground tissue morphologically and not to cortex (Fig 4 A). Casparian strips is a device crucial for selective nutrient uptake, exclusion of pathogens to the vascular cylinder (Naseer *et al*, 2012) and gives mechanical strength to the binding nature of endodermis around the stele. Similar to cork, the protective outer layer of stem/root, endodermis also gets suberised.

The presence of **amphicribral vascular bundles in the rhizomes of all the 4 species of *Curcuma* and of *Zingiber officinale* is worth consideration**. Such vascular bundles were reported earlier in primitive plants like ferns such as *Selaginella* and *Pteris* and in aquatic monocot*, Hydrilla.* But placenta of numerous taxa of angiosperms like Papaveraceae, Leguminosae, Winteraceae, Solanaceae, Gesneriaceae, Buxaceae, Annonaceae, Actinidiaceae, and Magnoliaceae etc and Kiwi fruit (Guo, X. *et al*.,2013) are found to contain amphicribral vascular bundles. These taxa cover the whole scope of angiosperms from the basal clade Magnoliids to the terminal eudicot lineage in the phylogenetic tree of APG (2009),(Guo, X. *et al*, 2017). In dicots such as *Begonia, Mesembryanthemum, Rheum,* and *Rumex*, amphicribral bundles occur as medullary bundles, which run through the pith (Mauseth, 1988). Amphicribral bundles are definitely a primitive feature because of their occurrence in pteridophytes and primitive monocots and their presence in placenta of higher plants. The presence of amphicribral vascular bundles in placenta of many plants indicates their utility in conducting large amounts of food to the developing ovules (to seeds) in fruits. As the phloem tissues are placed at the outer parts of the vascular bundles, food from these cells can directly be channelled to the seeds. It should be for the same reason the underground storage organs such as such as rhizomes of *Curcuma* and *Zingiber* retained this primitive character.

Conclusions

Secondary thickening in Monocots, mostly palms and rhizomatous species, is considered due to a Secondary thickening meristem which is now named as Monocot cambium which originates from pericycle

and or endodermis. The present study on four species of Curcuma, i.e. *C. amada, C. aromatica, C. caesia* and *C. longa* as well as *Zingiber officinale* proves the monocot cambium is exclusively pericyclic in origin. The cursory term "abnormal secondary growth" in monocots should be avoided as the secondary growth is always from pericycle and from evidences from molecular data on 80% overlapping of the regulatory principles of cambium regulation between Dicots and Monocots. The present study proves that Endodermis is better considered as the outer membrane of stele, than the innermost membrane of cortex. The prevalence of amphicribral vascular bundles as a primitive character retained in monocots also is suggested.

Authors' Contributions

The contributions of authors to the manuscript are as below
Mr. Rudra Patel: Procurement and sectioning of the specimen
Prof. M. Daniel: Conceptualization of the paper, Anatomical analysis and writing of the draft
Dr. Elizabeth Robin: Writing, Reviewing and editing the draft
All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

There is no ethical approval needed as animal samples and human samples were not involved in this experiment and manuscript..

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Conflict of Interests

"The authors declare that there are no conflicts of interest related to this article".

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