STUDIES ON THE VASCULAR CAMBIUM AND ITS DERIVATIVES IN SOME PRIMITIVE ANGIOSPERMS OF SUBTROPICAL MOIST FOREST OF MEGHALAYA

ABSTRACT

By

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THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
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Thesis

ABSTRACT

Angiosperms are the most dominant and successful land plants; they are distinguished by the presence of a flower (Maddison 2001). The primitive angiosperms were believed to be small trees or woody shrubs with large complex flowers (Taylor and Hickey 1996). The development of the first terrestrial plants coincided with the evolution of a vascular system that could transport water and solutes and also provide structural support.

Procambium and cambium represent two developmental stages of the meristem in plants; in the primary plant body the vascular system arises from the procambium while the secondary growth takes places due to the activity of the vascular cambium and give rises to secondary vascular tissues outside the primary ones. The development and evolution of vascular system in the land plants is the concomitant transition from the aquatic to the terrestrial habitat during the course of the evolution.

Unlike the apical meristems, which consist of a population of similar cells, the cambium has typically two morphologically distinct cell types, or initials; the axillary elongated fusiform initials leading to the axial system (including tracheids, vessel elements, fibres, axial parenchyma cells, sieve elements and companion cells) and the smaller isodiametrical ray initials giving rise to the radially orientated parenchymatous rays (Iqbal and Ghouse 1990).

The vascular cambium has been the subject of intense study ever since its importance in building up the plant body was felt. These works

described the seasonal changes in the vacuolation pattern in the fusiform initial and also elucidated the mechanism of additive and multiplicative cell division in the cambium. Bailey (1920a,b,c; 1923) made an accurate observations on the structure and dimensional changes in the vascular cambium in both the dicotyledons and gymnosperms, which were latter confirmed by using sophisticated instrument.

North-Eastern India is one of the megabiodiversity centres of the world (Swaminathan 1991; Mohan Ram and Seghal 2001). According to Takhtajan (1981), Indo-Malaysian tract is the cradle of the origin of Angiosperm. This region contains most of the arborescent and primitive angiospermous plants. However, the tree growth, particularly primitive angiosperms of sub-tropical moist forest of Meghalaya has not been studied in detail.

Therefore, in this thesis, three Brevi-deciduous species viz. *Dillenia indica* Linn. (Dilleniaceae), *Michelia champaca* Linn. and *M. oblonga* Wall. Ex Hk. f. (Magnoliaceae) and two Evergreen species viz. *Exbucklandia populnea* (R. Br. Ex Griff.) R. W. Br. (Hamamelidaceae) and *Magnolia grandiflora* Linn. (Magnoliaceae) were studied with reference to the following aspects.

- To find out the cambial activity in relation to phenology and climatic factors
- To study the seasonal variation in the structure, cytology and ergastic substance of the vascular cambium
- Duration of wood production

- Dimensional changes in the vascular cambium and its derivatives during different seasons

The interrelationship between different phenological events such as emergence of new leaves and buds, flowering, fruiting, seed dispersal and leaf fall, with environmental factors such as temperature, rainfall, relative humidity and periodicity of cambial activity was examined for all the five plants. *It was observed that there was a clear cut correspondence between* timing of vegetative bud break and cambial reactivation in all the five plants. Since the timing of flowering overlapped with the formation of new leaves, it could not be categorically proved that reproductive phase was really responsible for enhance activity of the cambium in these five primitive angiospermous trees.

It was observed that both cambial reactivation and peak activity were generally favoured by mean minimum temperature in *D. indica* and by mean temperature in rest of the four plants viz. *E. populnea, M. grandiflora, M. champaca* and *M. oblonga*; a positive correlation also existed between the least cambial activity/dormancy and lower mean temperature. Relative humidity had no effect on cambial periodicity. The selected plants did not have any water stress because they are growing in the sub-tropical wet forests of North east India, where water was not at all a limiting factor. Moreover, this study site is located 60 km from Cherrapunji, which is the region of highest rainfall.

The vascular cambium of all the five plants is of non-storied type. The length of fusiform initials was highest in *Exbucklandia populnea* and lowest in *Michelia champaca*. The radial and the tangential wall of fusiform initials were primary in nature; however, the radial wall was not only thicker than the tangential wall but also always beaded in nature. The beading was more prominent and closer to one another during dormancy than the active period of vascular cambium. The fusiform initials of all plants showed multinucleate condition 2-3 in *D. indica, E. populnea* and *M. grandiflora,* 3-4 in *M. champaca* and *M. oblonga,* of varied shapes and sizes. Cambial reactivation was marked by radial swelling of cambial cells, resulting in increase of width of cambial zone. This was followed by periclinal division and as a result of which the number of layers in the cambial zone increased. There was also an increase in length of fusiform initials which consequently brought about the thinning of cell walls so that the beads in the radial walls were not distinct. The frequency of periclinal divisions was more during the active state of the vascular cambium, while towards dormancy the cambial zone exhibited predominantly anticlinal divisions in the plants.

In all these primitive angiospermous trees, the secondary xylem were diffuse porous wood. The end walls of the vessel elements in all the plants had a scalariform perforation plate. Growth ring was distinct and demarcated by initial parenchyma in *M. champaca* and *M. oblonga,* by terminal parenchyma in *M. grandiflora.* But the growth ring is indistinct, occasionally indicated by layer of radially compressed fibres in *E. populnea,* delimited by smaller vessel and denser fibrous tissue in *D. indica.* Fibres tracheid and nucleated xylem fibres was present in *E. populnea.*

The duration of xylem production was about eight and half months in *E. populnea* and *M. champaca*, about eight months in *D. indica,* about seven and half months in *M. grandiflora* and *M. oblonga.* The length of fusiform initials and their derivatives like xylem fibres and vessel elements was maximum during the peak activity of the vascular cambium and was minimum during cambial dormancy. The trend in variation in the length of different vascular elements followed closely the trend in variation in length of fusiform initials from which they were derived.

Conspicuous changes could be found on the content of secondary metabolites (ergastic substances) during the approach of dormancy as well as during cambial reactivation and peak cambial activity. In *D. indica,* starch grain, polyphenol and tannin content were the major reserve products. But irrespective of the active and dormant cambium phenolic contents were present in *D. indica.* In *E. populnea,* the amount of starch grain, phenolic contents and crystal of calcium oxalate (cystolith) appeared in large quantity with the approach of dormancy but disappear slowly with the onset of cambial reactivation and disappear in the active period. The starch was the major food reserve in all the investigated plants viz. in *D. indica, E. populnea, M. grandiflora, M. champaca* and *M. oblonga*. Probably starch and crystal of calcium formed the source material for the new cell wall synthesis (e.g. Carbohydrates and calcium pectate) when the cambial derivatives are rapidly produced.

It was observed that the phenological events especially sprouting of new leaves and buds had a close relationship with cambial reactivations. The relationships of different climatic factors (monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity) with that of cambial parameter (the average width of cambial zone, the average width of differentiating xylem zone, the average length of fusiform initials, xylem fibres and vessel elements) were high explaining more than 40% of the variability between two variables. Therefore, multiple (partial) regression analysis was employed to see how much every climatic parameter contributes to the variability of the activity of the vascular cambium. Multiple (partial) regression analysis have shown that monthly mean minimum temperature in *D. indica* and mean temperature in *E. populnea,* *M. champaca, M. oblonga* and *M. grandiflora* is an important factor for cambial reactivation and xylem production. Further activity of vascular cambium and differentiation of xylem occurred due to the synergistic effect of both temperature and precipitation. Relative humidity had little/no effect on cambial activity and xylem formation. Cambial dormancy was imposed by the fall in mean temperature.

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Dedicated to

My

Beloved parents

THE NORTH-EASTERN HILL UNIVERSITY

SHILLONG

OCTOBER 2007

DECLARATION

I, M.G. Liangkuwang, here by declare that the subject matter of the thesis entitled **"Studies on the vascular cambium and its derivatives in some primitive angiosperms of subtropical moist forest of Meghalaya"** is the record of the work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University, for the award of the degree of Doctor of Philosophy in Botany.

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Place: Shillong

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(M.G. Liangkuwang)

CONTENTS

CHAPTER - 1

Introduction

Angiosperms are the most dominant and successful land plants; they are distinguished by the presence of a flower (Maddison 2001). The primitive angiosperms were believed to be small trees or woody shrubs with large complex flowers (Taylor and Hickey 1996). Recent re-evaluation of angiosperm evolution suggests that the ancestral condition might have been characterized by woody growth (Feild et al. 2004), consistent with previous speculation that extant herbaceous angiosperms had woody progenitors (Barghoorn 1965).

The development of the first terrestrial plants coincided with the evolution of a vascular system that could transport water and solutes and also provide structural support. According to the fossil records, terrestrial vascular plants identified at the end of Silurian and at the beginning of the Devonian era (*Cooksonia, Zosterophyllum, Psilophyton, Rhynia*) were without cambium. The cambium may have appeared between the lower Devonian and middle Devonian, but its presence was obvious from the upper Devonian (Kenrick and Crane 1997; Lauchad et al. 1999). The first known plant with a vascular cambium which exhibited true secondary growth was a species of free-sporing plant *Protopteridium*. The vascular cambium, allows the development of tree like forms and even the gigantism of some species (*Sequoia gigantia, Eucalyptus marginata*). Thus, the occurrence of the cambium has been a major event in the history of land plants.

Procambium and cambium represent two developmental stages of the vascular meristem in plants; and in shoots the procambium arise as a continuous cylinder, the procambium give rise to vascular cambium (Esau 1965b; Larson 1994). Procambial cells are recognized on a histological basis by their elongated, narrow shape, unvacuolated and darkly stained cytoplasm (Esau 1965b). In species lacking secondary growth, the procambial strands are said to be "closed" or determinate and in the species with secondary growth, procambial strands are "open" or indeterminate (Esau 1965b; Larson 1994).

The procambium, originating immediately below the shoot apex produces primary xylem and phloem (Ye 2002). The procambial cells form strands and form connections between newly developed organs with the conductive systems of the rest of the plant. The cells of the procambium differ from the surrounding parenchyma in that they are elongated, a key initial step in the differentiation into xylem or phloem cells (Ye 2002). The cambium arises at about the time internodal elongation is completed, and divisions in this meristem produce the secondary vascular tissues. It is clear that the sites for procambial cell initiation determine the pattern of vascular organization and that the activity of procambial cells controls the differentiation of vascular tissues.

The exact cellular organization of the vascular cambium has been subject of a longstanding debate, which is still not completely resolved to date (Larson 1994). The main controversy is concerned with the existence and position of a cambial initial. According to Hartig (1853), the cambium was formed by two cell layers, the outer one giving rise to the phloem and the inner one to the xylem. This idea was rejected by Sanio (1873), who proposed the uniseriate theory and considered that the cambium was made up of only one layer. This initial would divide continuously to produce xylem and phloem mother cells, which in turn divide only once or twice before proceeding to maturation. Besides establishing the so called "Sanio's Laws" governing the variation in the dimensions of the cambial derivative within a tree (Bailey and Shephard 1915), Sanio was responsible for the detailed description of the sequences of derivation of xylem and phloem cells from the uniseriate cambial initial layer. The group of four cells, consisting of an initial, mother cell and two daughter cells, first observed by Sanio, is now known as "Sanio's Four" (Mahmood 1968).

This strict view of a single "cambium" layer led to some controversy over whether the cambium is uniseriate or multiseriate (Larson 1994). Raatz (1892) and others believed in a multiseriate structure of the cambium. Not surprisingly, there are corresponding differences in the terminology used to identify the different layers of the cambium. One of the reasons for the controversy lies in the difficulty of identifying cambial initial as there is no clear difference in anatomy or ultrastructure between the proposed initial and the adjacent mother cells (Larson 1994). There is general agreement that more than one cell in the cambium is capable of division. It is further generally accepted that only the initial is capable of undergoing anticlinal divisions to initiate new cell files.

Unlike the apical meristems, which consist of a population of similar cells, the cambium has typically two morphologically distinct cell types, or initials; the axillary elongated fusiform initials leading to the axial system (including tracheids, vessel elements, fibres, axial parenchyma cells, sieve elements and companion cells) and the smaller isodiametrical ray initials giving rise to the radially orientated parenchymatous rays (Iqbal and Ghouse 1990).

The vascular cambium has been the subject of intense study ever since its importance in building up the plant body was felt. These works described the seasonal changes in the vacuolation pattern in the fusiform initial and also elucidated the mechanism of additive and multiplicative cell division in the cambium. So accurate were the observation of Bailey (1920a, b, c; 1923) that latter examination of cambium with sophisticated technique and instrument confirmed his description admirably. In the recent past, our knowledge of cambial cytology has been greatly extended by the several studies using the technique of transmission electron microscope (Srivastava 1966; Srivastava and O'Brien 1966; Kidwai and Robards 1969; Evert and Deshpande 1970; Murmamis 1971, 1977; Barnett 1973, 1975; Timell 1973, 1979, 1980b; Catesson 1974; Farooqui and Robards 1979; Barnett 1992; Mellerowicz et al. 1995; Farrar and Evert 1977a, b; Chaffey et al. 1997b; Oda and Hasezawa 2006). More information has been provided on the seasonal changes in the cambial activity in relation to climatic factors, the mechanism of cell plate formation and its extension and the changes during differentiation of vascular elements.

In plants undergoing secondary growth, increases in the diameter of woody stems are primarily due to activity of the vascular cambium (Larson 1994). The activity of the vascular cambium produces an uninterrupted cylinder of secondary vascular tissue (Chaffey 1999b; Dickison 2000). Periclinal divisions of fusiform initials produce xylem and phloem mother cells that in turn divide to produce xylem and phloem cells. Anticlinal divisions of fusiform initials lead to circumferential expansion of the cambium. Fusiform and ray derivatives may divide several times before differentiating into vascular tissues (Lachaud et al. 1999; Mellerowicz et al. 2001).

Esau (1965b) offers a terminological distinction between divisions in the cambium that increase the number of cells in the cambium (multiplicative divisions) and divisions of cambial initial that yield phloem to the outside and xylem to the inside (additive division). Xylem mother cells always divide more frequently than phloem mother cells, which explain the disproportion in size between phloem and xylem tissues. Once, the polarity of the cambium derivatives is established, the newly formed xylem mother cells undergo a highly ordered developmental process involving cell division, expansion, secondary cell wall synthesis/deposition, lignifications and programmed cell death (Fukuda 1996; Plomion et al. 2001; Ye 2002; Nieminen et al. 2004).

The rate of cambial growth is the major determinant for the production of wood in forest trees and it is determined by both the radial number of dividing cells in the cambial zone and the rate of cell division for each of the cambial zone cells (Gregory and Wilson 1968; Gregory 1971). Cambial growth is adjusted to the demands of water transport required by the leaf biomass and to provide the mechanical strength necessary to support the crown and to withstand wind forces (Zimmermann and Brown 1971).

Wood formation is a highly dynamic process; the onset, rate and duration of cell differentiation change during the growing season, creating a complex time–space system of xylogenesis (Wodzicki 1971; Uggla et al. 2001). The process of wood formation follows a pattern that is genetically determined and precisely regulated by gene expression (Hertzberg et al. 2001; Schrader et al. 2004), hormonal signals (Uggla et al. 1998; Schrader 2003) and environmental factors (Denne 1971, 1976; Savidge 1996; Antonova and Stasova 1997; Deslauriers and Morin 2005). Water, nutrients, temperature, photoperiod, gravity, wind, load and chemical substances are common factors that influence wood formation (Zimmermann and Brown 1971; Zobel and van Buijtenen 1989; Larson 1994; Chaffey 2002a).

The seasonal variations of cambial activity and annual rhythm of xylem and phloem differentiation in temperate, tropical, semi-arid and arid regions have been studied in quite a number of plants (Coster 1927,1928; Chowdhury 1939, 1940, 1941; Koriba 1958; de Alvim 1964; Fahn et al. 1968; Amobi 1974; Ghouse and Hashmi 1979; Denne and Dodds 1981; Dave and

Rao 1982a, b; Iqbal and Ghouse 1980, 1982, 1983, 1985a, b, 1987; Iqbal 1979, 1995; Venugopal 1986; Ajmal and Iqbal 1987a, b, 1992; Venugopal and Krishnamurthy 1987; Creber and Chaloner 1990; Borchert 1999; Priya and Bhat 1999; Rajput and Rao 2000b, 2001; Ogata et al. 2001; Rao and Rajput 2001a, b; Samuels et al. 2002; Yadav et al. 2002; Callado et al. 2004; Rajput et al. 2004, 2005; Schmitt et al. 2004; Akkemik et al. 2006; Heinrich and Banks 2006; Marcati et al. 2006; Pumijumnong and Wanyaphet 2006; Yanez-Espinosa et al. 2006; Venugopal and Liangkuwang 2007). In spite of these studies, there is a lacuna in the knowledge on cambial activity, organization, cytology and duration of wood production of trees growing in sub-tropical moist forest.

North-Eastern India is one of the mega biodiversity of the world (Swaminathan 1991; Mohan Ram and Seghal 2001). According to Takhtajan (1981), Indo-Malaysian tract is the cradle of the origin of Angiosperm. This region contains most of the arborescent and primitive angiospermous plants. However, the tree growth, particularly primitive angiosperms of sub-tropical moist forest of Meghalaya has not been thoroughly studied.

Therefore, in this thesis, three Brevi-deciduous species viz. *Dillenia indica* Linn. (Dilleniaceae), *Michelia champaca* Linn. and *M. oblonga* Wall. Ex Hk. f. (Magnoliaceae) and two Evergreen species viz. *Exbucklandia populnea* (R. Br. Ex Griff.) R. W. Br. (Hamamelidaceae) and *Magnolia grandiflora* Linn. (Magnoliaceae) were studied with reference to the following aspect.

1. To find out the cambial activity in relation to phenology and climatic factors
2. To study the seasonal variation in the structure, cytology and ergastic substance of the vascular cambium
3. Duration of wood production
4. Dimensional changes in the vascular cambium and its derivatives during different seasons

CHAPTER - 2

Review of Literature

In arborescent gymnosperms and dicotyledons, two stages may be discerned in the establishment of vascular tissue. The first stage is concerned with the formation of vascular tissue in the primary plants body; which is the product of apical meristem of roots and shoots. The second stage usually called secondary growth follows the first and result in increase in girth of the axial organs due to the production of more vascular tissue outside the primary ones. Secondary growth is due to a lateral meristem, the vascular cambium and displays an annual cycling pattern starting with activation of vascular cambium in the beginning of the growing season and stalling with dormancy of the cambium at the end of the season. The secondary growth in trees involves a series of sequential biological events, including maintenances of meristem cell entity, cell division, cell fate determination, differentiation, cell enlarging, secondary wall thickening, cell aging and cell death. Secondary growth in trees is annually regulated by seasonal changes, which leads to the formation of two kinds of wood (earlywood, which forms in early growing season and the latewood formed in late growing season) with distinctive structure and chemical compositions.

The cambium is of great significance, particularly in respect to the wood that is produced. Wood is a major terrestrial biomass and one of our most important natural materials (Plomion et al. 2001). The amounts of wood production by a tree depend on the activity of cambium and its duration during the growth period. Cambial activity ensures the perennial life of trees through the regular renewing of functional xylem and phloem.

A full understanding of the structure and function of the vascular cambium is a prerequisite for complete understanding of the growth and development of woody plants (Philipson et al. 1971; Barnett 1981; Iqbal and Ghouse 1990; Catesson 1994; Larson 1994). Much of the present knowledge on the cambial structure and function, and particularly the concept of cambial initial, arose from research on dormant cambial cells (Larson 1994). On the basis of the arrangement of cell ends of fusiform initials seen in tangential section, storied and non-storied types of cambium are recognized (Bailey 1920c). The majority of tree species have non-storied cambia, but many exhibit greater organization with their fusiform initials forming tangentially ordered ranks of storied cambium (Farrar and Evert 1997b). Storyed cambium can lose and regain its storied organization, but how organization may be induced and maintained is unknown. In non-storied cambium, ends of fusiform initials lie at different heights (Philipson et al. 1971; Romberger et al. 1993; Iqbal 1994, 1995; Larson 1994), whereas in the storied cambium, groups of fusiform initials form horizontal tiers with their ends terminating almost at the same height (Butterfield 1972; Ghouse and Yunus 1974a; Hejnowicz and Zagorska-Marek 1974; Krawczyszyn 1977; Yunus et al. 1978; Cumbie 1984; Iqbal and Ghouse 1990; Wloch and Polap 1994).

The storied nature of the cambium was initially assumed to have emanated from a storied procambium (Meeuse 1942; von Hohnel 1884).

However, later studies have shown that the storeyed cell arrangement of procambium may be lost in some species during the formation of the cambium (Record 1919; Carlquist 1988). On the other hand, formation of storied cambium from a non-storied procambium is related to radial longitudinal divisions (= longitudinal anticlinal divisions) of the fusiform initials which, on repeated occurrence would form packets of cells ending more or less at the same height (Timell 1980; Cumbie 1984; Larson 1994).

Recently, it was confirmed that intrusive growth between tangential walls is a basic event responsible for rearrangement of the initial cells (Kojs et al. 2004a, b). The mechanism of cell rearrangement was found to involve not only the occurrence of anticlinal cell divisions, but also a concomitant intrusive growth of the ends of cambial cells of one cell packet along the tangential walls of cells of the neighbouring packet (Kojs et al. 2004a, b). Kojs et al. (2004b) proposed that intrusive growth of the fusiform initials facilitated the formation of storied cambium during the early phase of the cambial development.

The vascular cambium comprises both fusiform and ray cells in almost all species, however in *Boerhaavia* species (Rajput and Rao 1998) and in *Trianthema monogyna* (Rao and Rajput 1998), rays were absent. The vascular cambium is distinct from apical meristems by having cells of variable size and shape as well as by having distinctive vacoulate protoplasm within the fusiform initials (Bailey 1920b, c; Larson 1994; Chaffey et al. 1997b; Farrar and Evert 1997a). The fusiform initial are elongated cells with tapered ends and the ray initial which are much smaller and which are almost isodiametric.

The polar, basipetal auxin flow is thought to play a role in the interconversion between ray and fusiform initials (Lev-Yadun and Aloni 1991). Fusiform initials to ray initials ratio, the seriation value of rays (uniseriate, biseriate and multiseriate), positioning of rays in relation to the apical and basal tips of fusiform initials, overall ray frequency, fusiform cell length, and so on, clearly are not rigidly determined because they vary with position and age of cambium (Bailey 1920a, b; Bannan 1951; Bannan and Bayly 1956; Iqbal and Ghouse 1990). When measured on tangential sections, it varies from 25% in a few tropical trees to 90 % in *Abies concolor* and exceptionally, 100 % in species of *Alseuomia* and *Bougainvillea* (Larson 1994; Iqbal 1995).

Besides, playing a role in the transmission of messenger molecules, rays are essential to the translocation of nutrients between phloem and xylem (Van Bel 1990; Barnett 1995). The rays have been considered to be "living bridge" (Catesson 1990). The high density of plasmodesmata within the pits of the tangential walls of xylem ray cells (Sauter and Kloth 1986), cambial ray cells (Goosen-de Roo 1981; Larson 1994; Farrar and Evert 1997a), and phloem ray (Farrar and Evert 1997b) has reinforced the ideas that the ray probably represent the major symplastic transport route for the exchanges of solute between the phloem and xylem (Van Bel 1990). A role of xylem ray cells as storage sites for reserve material during dormancy of temperate trees is also well documented (Sauter and Van Cleve 1990; Holl 2000; Sauter 2000).

Information on the length of cambial cells has been recognized as important in different aspect of botanical research, particulary, in plants taxonomy and phylogeny as well as in developmental aspect of ecological wood anatomy (Metcalfe and Chalk 1983). The dimension of fusiform initial length varies within species and they also vary positionally and seasonally within individual trees (Philipson et al. 1971; Iqbal and Ghouse 1990; Larson 1994). Many earlier studies on cambial cell length, involved species with storied cambium, in which cells are easier to measure in tangential sections (Ghouse and Yunus 1974a; Yunus et al. 1978; Ajmal and Iqbal 1987b; Siddiqi 1991; Rao et al. 1996). It has been suggested that the annual fluctuation in mean length of fusiform cambial initial might be due to changes in the frequency of anticlinal divisions (Swamy et al. 1960; Bannan 1967, 1970; Ajmal and Iqbal 1987a). The fusiform cells of non-storied cambium are usually longer than those of storied cambium (Metcalfe and Chalk 1983). Highly specialized dicotyledons with storied cambia have the shortest fusiform cells whose length (170-200 µm) does not vary greatly with age. On the other hand, the longest fusiform initials are found in gymnosperm and in dicotyledons with a non-storyed cambium.

It has been established that the dimensions and the morphology of the nuclei in cambial cells as well as their number of nucleoli vary within a single species according to both the season and the stage of cambial activity

(Mellerowicz et al. 1989; 1990, 1992; Lloyd et al. 1994, 1996). It is generally accepted that, each cambial initials contains a single nucleus (Bailey 1920a, b, c; 1930; Larson 1994). However, during the past 30 years there have been reports that the fusiform initials of some tropical species are multinucleate (Ghouse and Khan 1977; Iqbal and Ghouse 1987; Venugopal and Krishnamurthy 1989; Iqbal 1990; Larson 1994). Venugopal and Krishnamurthy (1989) report that, in the species they studied, multinucleate fusiform cells were present in active cambia but not in dormant ones. Seasonal variations in the number of nuclei have also been reported and in some species, fusiform initial containing five nuclei (Ghouse and Khan 1977).

However, in studies by transmission electron microscopy (TEM), no multinucleate cambial initials were found (Farrar and Evert 1997b; Chaffey et al. 1999). Recently, the cambial tissue of *Kalopanax pictus* were analysed by confocal laser scanning microscopy and observed that the cambial initials were exclusively mononucleate (Kitin et al. 2002). It has been suggested that a cambial cell might appear erroneously to be multinucleate under the microscope if two or more cells are super imposed in a tangential section (Larson 1994; Farrar and Evert 1997b). Nevertheless, it has been noted that, if multinucleate cambial cells do exist, they represent a curious phenomenon that necessitates detailed studies of the division of such cells, providing new avenues of research for cambial cytologist (Iqbal and Ghouse 1990; Catesson 1994; Lachaud et al. 1999). Whether cambial initials are mononucleate or multinucleate has not been resolved since to date.

Ultrastrastructure of cambial initials (Srivastava and O'Brien 1966; Kidwai and Robards 1969; Robards and Kidwai 1969; Catesson 1974, 1990; 1994; Rao and Dave 1983b, c; Rao and Catesson 1987; Fahn and Werker 1990; Barnett 1992; Kuroda and Sagisaka 1993; Rensing and Owens 1994; Rensing and Samuels 2004) and seasonal ultrastructural changes (Rao 1985; Sennerby-Forsse 1986; Farrar and Evert 1997a; Arend and Fromm 2003) have been extensively studied by transmission electron microscopy (TEM).

During the seasonal cambial activity, several changes occur in their structure and biochemistry (Riding and Little 1984; Mellerowicz et al. 1992a), cytoplasm (Farrar and Evert 1997a), nuclei (Mellerowicz et al. 1993; Llyod et al. 1996) and cytoskeleton cell wall continuum (Chaffey et al. 1997b). Cyclic cytoplasmic changes are well documented (Catesson and Lauchad 1993; Catesson 1994; Iqbal 1994, 1995; Larson 1994). The dormant cambial cells are filled with small vacuoles surrounded by organelles within a dense cytoplasm (Bailey 1930; Barnett 1973; Rao and Catesson 1987) while active cells are dominated by one or two large central vacuoles that confine the cytoplasm to narrow peripheral layers. Differences between vacuoles of initials in the quiescent and rest stages of dormancy were also found (Rensing and Samuel 2004).

The cytological details of periclinal and anticlinal division were described by (Bailey 1920b, c) including the oblique position of mitotic spindle and the two opposing phragmoplast, which stretch the newly formed cell wall down the central axis of the fusiform initials. Ultrastructural studies on cell division in the fusiform initials have carried out (Evert and Deshpande 1970; Goosen-de Roo et al. 1984; Farrar and Evert 1997b; Oribe et al. 2001; Chaffey and Barlow 2002; Rensing et al. 2002). The nuclei of the cambial initials generally maintain peripheral position until just prior to mitosis when a "phragmosome", a central bridge of cytoplasm, suspend the nucleus across the narrow radial width of the cell. Phragmosome were found to lie between the developing phragmoplasts and the lateral walls of dividing cambial initials (Evert and Deshpande 1970). However, longitudinal sections demonstrated that, extended phragmosomes did not traverse the axial length of fusiform initials (Farrar and Evert 1997b; Rensing et al. 2002). The nature of the phragmoplast, a cytoskeletal complex directing cell plate formation has been revealed by electron microscopy and immunofluorescence (Farrar and Evert 1997b; Chaffey and Barlow 2002).

Preprophase band (PPB) was not observed in dividing fusiform initials (Evert and Deshpande 1970; Farrar and Evert 1997b; Oribe et al. 2001; Chaffey and Barlow 2002). Cell division appears to take place in the absence of a preprophase band (ppb) in the fusiform initials (Farrar and Evert 1997b; Oribe et al. 2001; Chaffey and Barlow 2002; Rensing et al. 2002). The fact that fusiform initials divide in an ordered manner in the absence of preprophase band (ppb) not only demands further study, but is likely to cause us to re-evaluate our views on the role of the preprophase band (ppb) (Chaffey 2002d).

Occurrence of intrusive growth is believed to be essential after each oblique anticlinal division so as to enable the shortened sister fusiform initials to regain their original length (Bannan and Whalley 1950; Hejnowicz 1961; Iqbal 1994). Oblique anticlinal (pseudotransverse) divisions of a non-storied cambium are connected strongly with intrusive growth (Iqbal 1990, 1995; Larson 1994; Kojs et al. 2004b). Recently, it was demonstrated that intrusion, of the fusiform initial takes place along tangential walls of adjacent fusiform initials and their immediate derivatives (Kojs et al. 2004a, b; Jura et al. 2006). It was generally thought that circumference of a storied cambium increases because of tangential growth of newly produced cells after each radial longitudinal division, whereas that of a non-storied cambium increases because of intrusive cell growth between radial walls of the daughter initial after each pseudo-transverse division (Whalley 1950; Philipson et al. 1971; Harris 1989; Romberger et al. 1993; Larson 1994). This means that the increase in circumferential growth of storied cambium and non-storied cambium occurs by two different mechanisms. However, Jura et al (2006) observations indicate that, in both cases, it is due to a symplastic growth in tangential direction and apical intrusive growth, which is responsible for cell elongation, occurs between tangential walls of adjacent cell layers of the cambium and does not contribute to the increase in the cambial circumference. The only difference between the intrusive growth of fusiform initials in the storied and the non-storied cambia is the varied extent of this growth. The intrusive growth between tangential walls is a basic event responsible for rearrangement of the initial cells (Kojs et al. 2004a, b).

Secondary xylem or wood develops by a succession of four major steps, including cell division, cell expansion, cell wall thickening, and programmed cell death (Chaffey 1999b; Mellerowicz et al. 2001; Plomion et al. 2001; Aspeborg et al. 2005; Li et al. 2006; Samuels et al. 2006). Through periclinal divisions of cambial initials, secondary xylem develops inwards or centripetally and secondary phloem develops outwards or centrifugally (Niklas 1999; Chaffey 2002a; Helariutta and Bhalerao 2003). Cambial derivative production and subsequent phloem and xylem cell differentiation sometimes occur concomitantly on opposing sides of the cambial zone, but usually, when one side is developing the other appears to be awaiting its turn, and it remains unclear what controls whether the centripetal or centrifugal side is favoured (Larson 1994).

Following cell division, cambial cell derivatives undergo a period of cellular expansion. Variation in differentiated xylem cells begins immediately at this early stage of development. Angiosperm vessels and gymnosperm tracheids undergo radial expansion while, fibres of angiosperms undergo intrusive elongation (Mellerowicz et al. 2001).

Once expansion is completed, the formation of the secondary cell wall begins with the biosynthesis and assembly of three major compounds; polysaccharides (cellulose and hemicelluloses), lignins and cell wall proteins (Plomion et al. 2001; Junghans et al. 2004). At the end of the cell-expansion phase, the protoplast begins to produce the thickened secondary cell wall, made of cellulose microfibrils (Donaldson 2001).

After deposition of the secondary cell wall, the tonoplast within the xylem cell ruptures, releasing proteases and nucleases from the vacuole that break down the cytosolic structures and induce the cell to undergo programmed cell death (Fukuda 2000; Obara et al. 2001; Ito and Fukuda 2002). The final stage of development for secondary xylem cells is autodigestion of their living protoplast. Cell death is initiated by the disruption of vacuole membranes that results in the release of hydrolytic enzymes into the cytosol (Groover and Jones 1999).

The ability of plants meristems to alternate between active growth and dormancy and the establishment of the dormant state play a key role in the survival of adverse environmental conditions. In most species, the cambium exhibits alternate active and dormant phases during a growth year. Radial and apical growth in most of the woody species is periodic and periodicity is clearly correlated with seasonal changes (Iqbal 1994). Periodicity of cambium and xylem production is controlled by various environmental and physiological factors. (Philipson et al. 1971; Kramer and Kozlowski 1979; Ajmal and Iqbal 1987a, b; Iqbal 1994; Larson 1994; Rao and Rajput 1999; Grotta et al. 2005; Gricar et al. 2006; Groover and Robischon 2006; Li et al. 2006; Yanez-Espinosa et al. 2006; Druart et al. 2007). The maintenance of cambial integrity depends on genetically controlled species-specific rhythm (Hejnowicz 1975; Stieber 1985). The effect of genetic and environmental factors on shoot growth and xylem formation has been studied (Longman et al. 1979). Coster (1928) concluded that the tendency of rhythmic growth is genetically fixed but the periodicity can be altered by the changing growing condition. These results were confirmed by Fahn and Werker (1990) and Koriba (1958).

In tropical climate, the cambium remains active throughout (Dave and Rao 1982a; Fahn 1982) or for major part of the year (Fahn and Sarnet 1963; Dave and Rao 1982b). However, not all tropical trees exhibit a continuous activity (Coster 1927, 1928; Mariaux 1967; Fahn et al 1981; Ash 1983). The tropical trees were thought of as plants with continuous growth throughout their life cycle, lacking growth boundaries due to imperceptible climatic variability and seasonality (Mariaux 1981; Detienne 1989). It has now been recognized that most trees growing in seasonal climatic regions have dormant periods in response to vascular cambium inactivity. Moreover, there is evidence of seasonal growth in trees distributed in non-seasonal environments (Killmann and Thong 1995). In temperate climatic regions, cambial activity is a periodic process, which usually occurs from late spring to late summer (Denne and Dodd 1981; Larson 1994; Savidge 1996, 2000; Lachaud et al. 1999; Wodzicki 2001; Funada et al. 2002; Larcher 2003; Gricar et al. 2006; Begum et al. 2007).

The resumption of cambial activity in spring is brought about by both internal chemical factors and external conditions (Savidge and Wareing 1981). Cambial reactivation was preceded by a partial loosening of the radial walls, especially in cell junction (Rao 1985; Funada and Catesson 1991). Nevertheless, the criteria for determining cambial reactivation are not yet generally accepted. A clear difference between diffuse-porous and ring porous hardwood trees, regarding the onset of cambium activity in spring was pointed out by Preistly and Scott (1936). Frankenstein et al. (2005) have concluded that for the ring-porous and diffuse-porous wood species, the most appropriate criterion of reference in determining cambium reactivation should be the first observation of cell division as suggested Farrar and Evert (1997b). With the resumption of cell-division, many small vacuoles filling the cambial cells coalesce, so in active cambial cells, the cytoplasm occupies a narrow peripheral layer surrounding a single large vacuole (Bannan 1955; Rensing et al. 2002), as well as the fusiform initials have very active cytoplasmic streaming (Thimann and Kaufman 1958). The prominent microfilament bundles and associated organelles are consistent with an actin–myosin system supporting this streaming is confirmed by immunofluorescence and transmission electron microscopy (Chaffey et al. 1997b; Samuels et al. 2002). The anatomical, cytological, biochemical and histochemical changes that occur during cambial reactivation have been studied (Barnett 1992; Farrar and Evert 1997a, b; Arend and Fromm 2003; Rensing and Samuels 2004). These studies reveal the general outline of the events that occur in cambial cells during natural reactivation. However, the physiological regulation of cambial reactivation in the spring is still not fully understood (Funada et al. 2002).

Cambial dormancy is an example of a massive switch in the physiological status of a tissue. During dormancy, the meristematic activity of the cambium is arrested in the G1 phase once cell division is terminated (Mellerowicz et al. 1989, 1992; Zhong et al. 1995). During the induction of dormancy, the central vacuole in the cambial cells disintegrates into several smaller vacuoles, necessitating the synthesis of new vacuolar membranes and membrane lipids (Farrar and Evert 1997a, b). The transition to dormancy also involves extensive changes in the cellular structure such as thickening of the cell walls and alterations in the vacuolar structure (Farrar and Evert 1997a, b). The execution of these physiological and metabolic changes during dormancy is regulated through a complex interplay between environmental and hormonal signals.

During the transition to dormancy, polar auxin transport is reduced and the cambium is rendered insensitive to applied auxin (Little and Bonga 1974; Schrader 2003). Both in conifers and dicot trees, cambial cell walls are thick during the dormant period and thin during the period of active growth (Catesson 1964; Riding and Little 1984; Funada and Catesson 1991; Chaffey et al. 1998). Riding and Little (1984, 1986) recognized periods of actively dividing cells, rest and quiescence stage during the annual cambial cycle. Efforts have been made to estimate the extent of cambial dormancy and to identify factors that maintained or break dormancy.

It has been long suggested that a relationship exist between bud break and cambial reactivation. Priestly (1930) was the first one to suggest that cambial activity in conifers and dicots normally starts at the base of apical buds. Most species showed unique times of growth initiation and cessation during the year (Hartshorn 1983; Reich and Borchert 1984; Janzen 1986; Murphy and Lugo 1986; Breitsprecher and Bethel 1990). Initiation of cambial activity in relation to bud breaks is not identical for all the plants types. In some species, such as *Prosopis spicigera* (Iqbal and Ghouse 1982) and *Streblus asper* (Ajmal and Iqbal 1987a), leaf emergence and subsequent cambial reactivation are separated by a gap of several weeks. In the deciduous conifer, *Larix leptolepis*, cambial reactivation occurs a few weeks after bud flushing (Oribe et al. 1993).

The phenological variation and environmental changes play an important role in the initiation, peak and cessation of cambial activity (Venugopal and Krishnamurthy 1987; Iqbal 1990; Paliwal and Paliwal 1990; Larson 1994; Rao et al. 1996; Rao and Rajput 1999). A close relation between cambial activity and new leaf formation was recorded in many tropical and sub-tropical belts (Iqbal and Ghouse 1985b, Iqbal 1990; Paliwal and Paliwal 1990). Amobi (1974) found that the initiation of the cambial activity was correlated with bud break and leaf formation. It has been reported that, the bud burst and development of new leaves are related to cambial reactivation and xylem differentiation (Aloni 1991; Suzuki et al. 1996; Ljung et al. 2001; Schrader 2003; Barlow and Powers 2005). In most of the tropical species, vascular cambium activity is mainly determined by the activity of apical buds (Creber and Chaloner 1990; Borchert 1999). Several studies have report, that the phenological behaviour and growth periodicity of tropical trees are influenced by different climatic factors, including day length (Frankie et al. 1974; Breitsprecher and Bethel 1990), internal water status, soil moisture and relative humidity (Reich and Borchert 1984; Williams et al. 1997; Schongart et al. 2002), thermoperiodic mechanism (de Alvim 1964) and temperature (Paliwal and Prasad 1970; Paliwal et al. 1975; Ghouse and Hasmi 1979; Iqbal and Ghouse 1982; Ajmal and Iqbal 1987b).

Studies inferring causal relationship among trees radial growth, phenology and local environmental factors have been carried out in recent years (Ghouse and Yunus 1974a; Fahn et al. 1981; Mariaux 1981; Rao and Dave 1981; Dave and Rao 1982a, b; Iqbal and Ghouse 1985a; Ajmal and Iqbal 1987a, b; Venugopal and Krishnamurthy 1987; Breitsprecher and Bethel 1990; Iqbal 1990; Borchert 1999; Priya and Bhat 1999; Rao and Rajput 1999, 2001a, b; Ogata et al. 2001; Rao and Rajput 1999, 2001a, b; Samuels et al. 2002; Yadav et al. 2002; Callado et al. 2004; Jones et al. 2004; Rajput et al. 2004, 2005; Schmitt et al. 2004; Deslauriers and Morin 2005; Akkemik et al. 2006; Heinrich and Banks 2006; Marcati et al. 2006; Pumijumnong and Wanyaphet 2006; Tardif et al. 2006; Yanez-Espinosa et al. 2006; Heinrichs et al. 2007; Venugopal and Liangkuwang 2007). The effect of flood on tree growth in the Amazon forest in relation to phenology and analysis of ring has been studied in detail (Worbes 1999; Schongart et al. 2002).

Physical factors of the environment act on the seasonal activity of the cambium by way of hormonal messengers (Lauchad et al. 1999). Environmental factors interact in a complex way under natural conditions, which complicates analysis of the effects of selected factors on xylogenesis. However, the effect of individual factors on xylogenesis has been successfully demonstrated in experiments with shoots, stem cuttings or intact stem portions growing under controlled conditions (Little and Bonga 1974; Savidge and Wareing 1981; Riding and Little 1984, 1986; Mellerowicz et al. 1992b; Barnett and Miller 1994; Oribe and Kubo 1997; Oribe et al. 2001, 2003, 2004; Wodzicki 2001; Rensing and Samuels 2004; Begum et al. 2005; Gricar et al. 2006; Begum et al. 2007). Among the studies that deal with wood formation in relation to environment there are few which observed differentiated phase of cell growth (Waisel and Fahn 1965; Fritts et al. 1991, 1992; Antonova and Stasova 1996; Wodzicki 2001).

Temperature has been proposed as the main factor initiating the onset of cambial activity (Ajmal and Iqbal 1987a, b; Iqbal 1990; Oribe and Kubo 1997; Rao and Rajput 1999, 2001b; Vaganov et al. 1999; Tardif et al. 2001a; Funada et al. 2002; Horacek et al. 2003; Yanez-Espinosa et al. 2006; Begum et al. 2007; Heinrichs et al. 2007). High temperature is necessary for leaf initiation and favouring cambial activity simultaneously (Rao and Rajput 1999; Yanez-Espinosa et al. 2006). A similar view was expressed by Kramer and Kolzowski (1979), that, the temperature was a significant factor for bud break following reactivation and subsequent shoot growth. Localized heating has been reported to induce cambial reactivation earlier than natural cambial reactivation (Savidge and Wareing 1981; Barnett and Miller 1994; Oribe and Kubo 1997; Oribe et al. 2001, 2003; Gricar et al. 2006; Begum et al. 2007). Savidge and Wareing (1981) also suggested that temperature was a limiting factor for cambial reactivation. An increase in temperature might induce the conversion of storage starch to sucrose for the activation of cambial cell division and secondary xylem formation (Begum et al. 2007). However, the response of dormant cambium of different species to the external application of heat was different (Barnett and Miller 1994; Oribe et al. 2001, 2003, 2004; Begum et al. 2007).

Besides temperature, rainfall has also a direct bearing on the enhancement of cambial activity (Kramer and Kozlowski 1979; Rao et al. 1996; Rao and Rajput 1999). The role of rainfall on cambial behaviour and xylem production under drought condition was studied much more intensively than other factors in the past. Higher rainfall was reported to be conducive to cambial reactivation in several plants growing especially in the tropic and semi-arid climates (Glock 1955; Reinders-Gouwentak 1965; Rogers 1981; Dave and Rao 1982a, b). Many studies have examined the relationship between tree water status and xylem development (Larson 1963; Zahner et al. 1964; Abe and Nakai 1999). The correlation between cambial growth and rainfall has been assessed in the lowland dipterocarp forest of Peninsular Malaysia (Killmann and Thong 1995).

Studies carried out in boreal forest of Quebec showed that, the early summer precipitation had positive effect on annual growth (Brooks et al. 1998; Dang and Leiffers 1989; Hofgaard et al.1999; Arehambault and Bergeron 1992; Hofgaard et al. 1999). Rainfall probably is an important factor only in the regions where soil moisture content is dependent on rainfall (Rao and Rajput 2001a, b). Studies on growth periodicity in the xylem of tropical and sub-tropical species in Brazil have shown that rainfall defines the periodicity of xylem production (Worbes 1985, 1989; Botosso and Vetter 1991; Luchi 1998).

Studies have shown that auxin is an important regulator on the cambial activity (Savidge and Wareing 1981; Little and Savidge 1987; Aloni 1995; Little and Pharis 1995; Uggla et al. 1998; Sundberg et al. 2000; Ye 2002). Auxin plays a key role in regulating wood formation through its effects on cambial activity and xylem development (Sundberg et al. 2000). After cambial reactivation, a continuous supply of IAA was needed for the maintenance of cambial cell divisions and cell development (Oribe and Kubo 1997; Sundberg et al. 2000; Oribe et al. 2003). It has been proposed that, the indole-3-acetic acid (IAA) is required for the maintenance of meristematic activity as well as the shape of fusiform initials and the radial enlargement of cambial derivatives (Savidge and Wareing 1981; Savidge 1983; Sunberg et al 2000). Auxin application enhances cambial activity and induces differentiation of secondary xylem and phloem (Savidge and Wareing 1981; Savidge 1983; Little and Savidge 1987; Little and Pharis 1995; Savidge

2001). The levels of endogenous IAA vary with differences in season and age of the tree (Sundberg et al. 1987, 2000; Funada et al. 2001).

Majority of past the studies on cambial activity and xylem production are pertained to plants growing in temperate region with definite seasonal climate (Bailey 1920a; Bannan 1955, 1962; Antonova 1996, Antonova and Stasova 1997; Rensing and Samuel 2004). However, studies on the seasonal activity of vascular cambium and production of xylem in relation to different climatic factors in sub-tropical wet forest are scarce.

CHAPTER - 3

Materials and Methods

Study Area and Climate

The upland area, where most of the sub-tropical mixed forest exists, was selected for the study in Botanical Survey of India, Experimental Garden, Barapani, Shillong (25° 34' N and 91° 53 ' E) with an elevation 1,100 m above sea level (Plate- 3.1). The elevation above sea level is characterized by mountain climate or wet hill forest climate with low temperature and relatively high precipitation (Lal 2000; Worbes and Junk 1989). As the altitude increases, various climatic elements such as pressure, temperature and precipitation undergo well-defined changes (Lal 2000). The soil is loamy, reddish brown in colour and lateritic in origin. The pH ranges from 5.9 to 6.2 (Singh 1996; Porwal et al. 2000; Mishra et al. 2003, 2004).

The data on the climatic factors were collected from Central Seismological and Meteorological observatory, Shillong station, Government of India as well as from Engineering division, ICAR Research complex, Barapani, Shillong. The climatic factors (monthly mean temperature, mean maximum temperature, mean minimum temperature, precipitation and relative humidity) for the years 2002 and 2003 were chosen for this study (Table 3.1 and Fig. 3.1).

Climatologically, this study area belongs to the sub-tropical wet climatic region (Champion and Seth 1968). This region received abundant southwest monsoon and during winter the region received northeast monsoon. On the basis of variation of temperature, rainfall and wind, the year in the region may be divided into four distinct seasons: (1) winter (December - February), (2) pre-monsoon or summer (March - May), (3) monsoon (June - September) and (4) retreating monsoon (October and November) (Porwal et al. 2000; Tripathi et al. 2004). Highest rainfall was recorded in the months of June and July i.e. above 480 mm and minimum rainfall were recorded from December up to February i.e. less than 50 mm. In winter the mean temperature ranges from 12 to 16°C and during summer the mean temperature range from 18 to 25°C.

Field Observations and Collections

The following plants were selected for the present study:

Brevi-deciduous species

1. *Dillenia indica* Linn. (Dilleniaceae)
2. *Michelia champaca* Linn. (Magnoliaceae)
3. *Michelia oblonga* Wall. Ex Hk. f. (Magnoliaceae)

Evergreen species

1. *Exbucklandia populnea* (R. Br. Ex Griff.) R. W. Br. (Hamamelidaceae)
2. *Magnolia grandiflora* Linn. (Magnoliaceae)

To study the cambial activity, the selected plants samples were collected from the plants growing in Botanical Survey of India, Experimental Garden, Barapani, Shillong. Periodic collection of twigs measuring from 1.5 to 2 cm in diameter was made from ten plants of the above mentioned trees at every fortnight interval for two consecutive years (2002-2003). The timing of different phenological events such as flushes of new leaves and buds, flowering, fruiting, seed dispersal and leaf fall were recorded during this study period 2002 and 2003. The plants samples were fixed in FAA (formalin (5ml): Acetic acid (5ml): 70 % Alcohol (90ml), 3 % Glutaraldehyde in 7.2 pH phosphate buffer and Cornoy's fluid in the field itself. The samples fixed in Cornoy's fluids were transferred to 70% ethanol for preservation.

Analyses in Laboratory

Because of the difficulty in sectioning the intact cambial tissue (Catesson 1974; Goosen-de Roo and Van Spronsen 1978), as it is sandwiched between the thick - walled xylem cell on one side and bast fibres on the other side, also as it is subjected to radial and tangential tension, the material to be sectioned was trimmed to small size using the method suggested by Newman (1956) and followed by Goosen-de Roo and Van Spronsen (1978). The suitably trimmed samples were dehydrated in a tertiary butyl alcohol (TBA) series, according to the method of Sass (1958) and embedded in paraffin (Berlyn and Miksche 1976) or glycol methacrylate (Feder and O'Brien 1968).

Paraffin or glycol methacrylate embedded material were sectioned in three planes; transverse section (TS), tangential longitudinal section (TLS) and radial longitudinal section (RLS) at a thickness of 8 to10 µm using Leitz rotatory microtome. These were supplemented with thin hand sections as well as sliding microtome. The sections were stained according to any of the following staining procedure:

1. Saffranin O and Fast Green (Johansen 1940).
2. Toluidine blue O (Feder and O'Brien 1968).
3. Lugol's Iodine (Johansen 1940).
4. Periodic Acid and Schiff's reagent (PAS) (Jensen 1962).
5. Methyl Green and Pyronin G (Jensen 1962).
6. Tannic acid and Ferric Chloride counterstained with lacmoid blue (Cheadle et al. 1953).
7. Acid fuschin (Feder and O'Brien 1968).

Total starch and phenol contents were localized by using iodine-potassium iodide and Gibb's reagent respectively (Johansen 1940; Gahan 1984; McCully 1966).

For micro measurement, recently formed xylem tissue were carefully teased out and macerated according to Jeffrey's method (Berlyn and Miksche 1976) for determining the dimension of different xylem element in all the months of the study period 2002 and 2003. The length and width of xylem fibres and vessel element were measured from the macerated wood samples. The numbers of cambial layers, width of cambial zone and differentiating xylem zone were measured from the transverse section (TS). The length and width of fusiform initials was measured from the tangential longitudinal section (TLS). In all the measurement, which were made with ocular and stage micrometers, a minimum of 100 reading were taken from each randomly chosen sample. Photographs were taken from Nikon E600 light microscope.

Data analysis

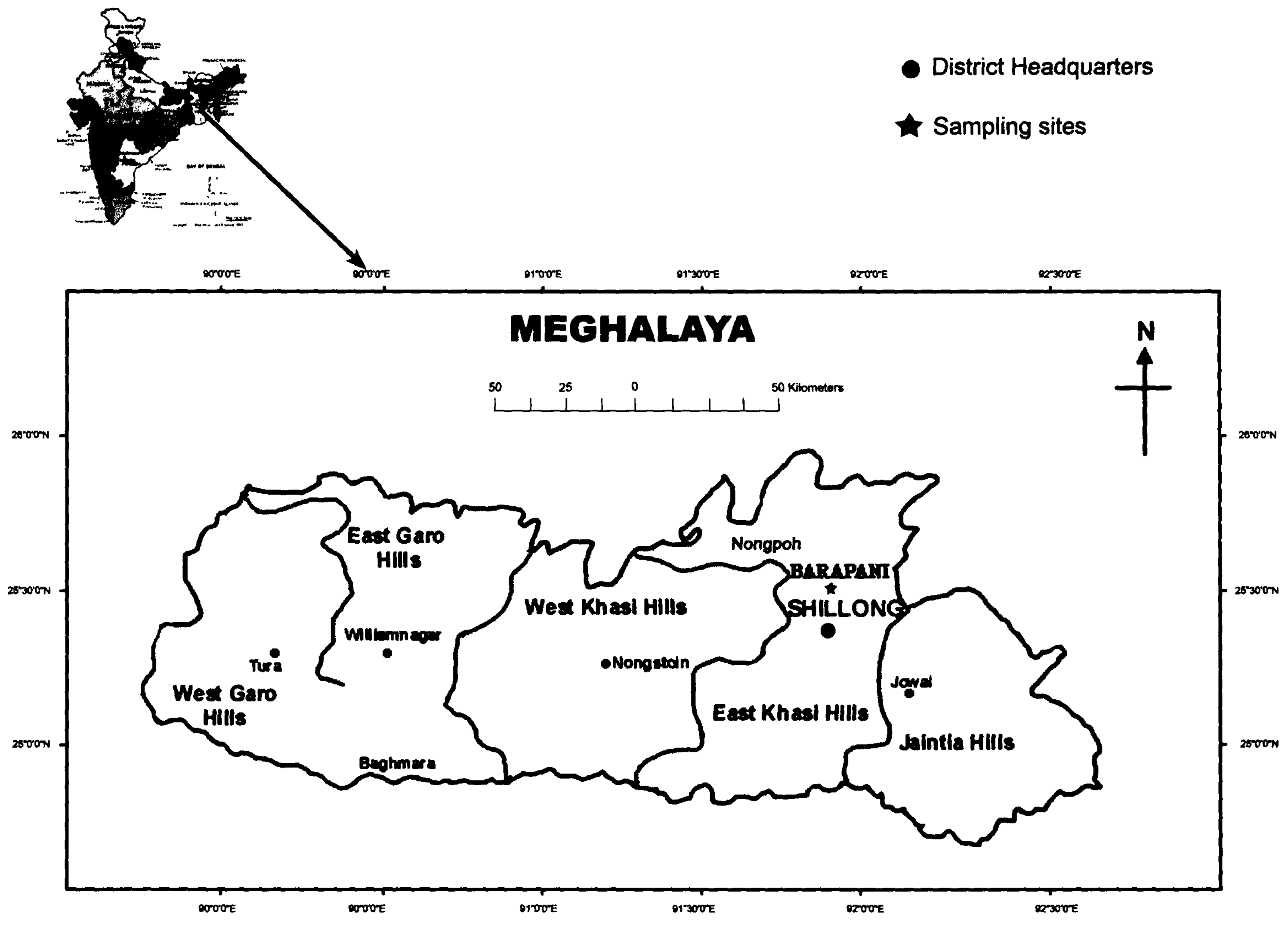
To study the role of climatic factors on the activity of the vascular cambium and xylem production on these selected plants, the following statistical methods were employed. The mean and standard deviation was calculated for all the measurement made. Statistical analysis of relationship between climatic factors (monthly mean, maximum and minimum temperature, rainfall, relative humidity) and anatomical variables (average width of cambial zone, average length of fusiform initials, average width of differentiating xylem zone and average length of xylem fibres and vessel elements) were calculated by using Karl Pearson's correlation coefficient and multiple (partial) regression analyses. Multiple (partial) regressions is used to assess the relationship between one dependent variable and several independent variables.

The goal of multiple regression analyses was to analyses the specific effect of particular independent variable when all other variables are held constant. The anatomical variables (average width of cambial zone, average length of fusiform initials, average width of differentiating xylem zone and average length of xylem fibres and vessel elements) were response or dependent variables and climatic factors (monthly mean temperature, mean maximum temperature, mean minimum temperature, monthly mean precipitation and monthly mean relative humidity) were regressors or independent variables. The values of (*t*) were compared to find out whether the relationship of each regressor with the dependent variable was statistically significant. Furthermore, this type of study is applicable to estimate tree's productivity and growth as well as evaluating past and present forest environments through tree ring research (Fritts 1976; Schweingruber 1988, 1996; Worbes 1995, 2002). All Statistical analyses were performed with STATISTICA version 5 software.

Plate -3.1

Map showing the sampling site.

Plate-3.1

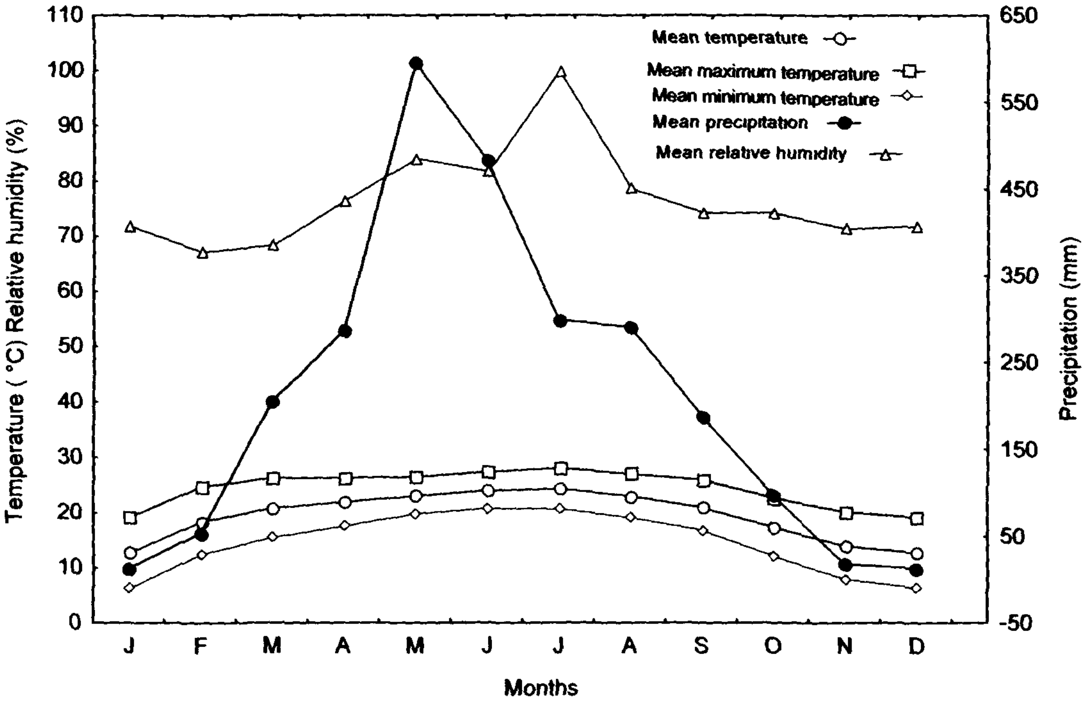


Map of Meghalaya in India showing sampling site

Table 3.1 Average climatic data of the years 2002-2003.

Months	Monthly mean temperature in °C.	Monthly mean maximum temperature in °C.	Monthly mean minimum temperature in °C.	Monthly mean precipitation In mm.	Monthly mean relative humidity in %.
January	12.76	19.20	06.37	13.19	71.90
February	15.46	21.86	09.16	19.28	70.73
March	18.43	24.80	12.49	53.56	67.20
April	20.91	26.36	15.54	205.02	68.40
May	22.00	26.40	17.63	286.80	76.46
June	23.12	26.57	19.85	595.06	84.16
July	24.10	27.49	20.83	482.50	81.80
August	24.40	28.17	20.74	298.90	81.50
September	23.08	27.08	19.16	290.26	80.90
October	20.95	25.98	16.84	188.06	78.70
November	17.40	22.74	12.13	97.73	74.30
December	14.06	20.26	08.01	18.06	71.43

Fig. 3.1 Graphical representation of average climatic data of the years 2002-2003.



CHAPTER - 4

Dillenia indica Linn.

Introduction

The family Dilleniaceae comprise of about 10 genera and approximately 350 species of trees, shrubs or woody climbers or rarely herbs. In India, the Dilleniaceae is represented by 6 genera, comprising of 30 species. The genus *Dillenia* consists of 15 to 20 species of mostly trees, rarely shrubs and widely distributed throughout the Indo-Malayan region and extends up to Australia. Seven species of *Dillenia* occurs in India and three species are timber yielding trees. *Dillenia indica*, commonly known as Elephant apple tree is a brevi-deciduous type growing up to 10m or more in height.

In *D. indica*, the relative proportion of ray initials in the main stem was 75% (Ghouse and Yunus 1974b). The structure of wood and its physical and mechanical properties of *D. indica* were described by (Pearson and Brown 1981).

The activity of vascular cambium is not uniform throughout the year and determined by the interaction of internal and external factors (Philipson et al. 1971; Larson 1994; Iqbal 1994; Grotta et al. 2005). The majority of the studies on cambial activity are pertained to plants growing in temperate region with definite seasonal climates (Bailey 1920a; Bannan 1955, 1962; Antonova 1996; Antonova and Stasova 1997; Rensing and Samuel 2004). The seasonal variations of cambial activity and annual rhythm of xylem and phloem differentiation in tropical trees, semi-arid and arid regions have been studied in quite a number of plants (Coster 1927, 1928; Chowdhury 1939, 1940, 1941; Koriba 1958; de Alvim 1964; Fahn et al. 1968; Amobi 1974; Ghouse and Hashmi 1979; Denne and Dodds 1981; Dave and Rao 1982a, b; Venugopal 1986; Venugopal and Krishnamurthy 1987; Creber and Chaloner 1990; Larson 1994; Borchert 1999; Priya and Bhat 1999; Rao and Rajput 2001a,b). The effect of genetic and environmental factors on shoot growth and xylem formation has been studied in the West African tropical tree (Longman et al. 1979). Periodicity of wood formation in twigs of 11 tropical trees was studied in different ecological areas, such as lowland rainforest, savannah and mangrove swamps in Nigeria (Amobi 1974). Recently, the wood production has been estimated in the natural forest stand in Cameroon by using tree ring analysis (Worbes et al. 2003). Cambial activity and annual rhythm of xylem development in trees and shrubs of desert plants have been studied in Israel (Fahn 1958). In the Southeast Asian countries, a sizable number of trees have been studied. The correlation between cambial growth and rainfall has been assessed in the lowland dipterocarp forest of Peninsular Malaysia (Killmann and Thong 1995). Cambial activity, development of xylem and phenology have been studied in *Azardiracta indica* (Rao and Rajput 2001b), *Tectona grandis* (Berlage 1931; Rao and Dave 1981; Venugopal and Krishnamurthy 1987; Priya and Bhat 1999; Rao and Rajput 1999), *Acacia nilotica* (Rajput and Rao 2000b, Rao and Rajput 2001a), *Albizzia lebbeck* (Venugopal and Krishnamurthy 1987), *Dalbergia sissoo* (Ghouse and Yunus 1974a; Venugopal and Krishnamurthy 1987), *Polyalthia longifolia* (Ghouse and Hashmi 1979), *Mangifera indica* (Dave and Rao 1982b; Venugopal and Krishnamurthy 1987), *Calophyllum inophyllum*, *Morinda tinctoria*, *Terminalia crenulata* (Venugopal and Krishnamurthy 1987).

On the basis of phenological rhythm, the tropical trees have been classified into four types (evergreen, stem succulent, deciduous and brevi-deciduous) growing under the same ecological conditions (Borchert 1999). The effect of flood on tree growth in the Amazon forest in relation to phenology and analysis of growth ring has been studied in detail (Worbes 1999; Schongart et al. 2002; Dezzeo et al. 2003). However, studies on the seasonal activity of vascular cambium and production of xylem in relation to different climatic factors in sub-tropical wet forest are scarce. Therefore, the present study on annual rhythm of cambial activity and differentiation of xylem in relation to phenology as well as climatic improves our further understanding of tree growth in *D. indica* growing in sub-tropical wet forest of northeast India. Furthermore, this type of study is applicable to estimate tree's productivity and growth as well as evaluating past and present forest environments through tree ring research (Fritts 1976; Schweingruber 1988, 1996; Worbes 1995, 2002).

Phenology

In *D. indica*, swelling of young vegetative buds was observed in the middle of April. By the middle of May, the maximum leaf flushing started and continued up to the end of December. Sprouting of new leaves as well as persistent mature leaves was observed from the middle of April to January.

Leaf senescence was initiated from the month of February, and the defoliation continued up to the end of March. The tree was completely barren for a brief period of 15 days; therefore, *D. indica* belongs to brevi-deciduous type (Borchert 1999).

Flowering was noticed in the months of June and July. The flowers are very large. Fruiting was observed in the month of August, and mature fruits were borne on tree from September to the last week of October. The dispersal of seeds took place in the months of October to December. The seeds were small and brown in colour. Different phenophases such as flushes of new leaves, flowering, fruiting, seed dispersal and leaf fall were given (Fig. 4.1).

Structure of vascular cambium and its activity

In *D. indica*, the vascular cambium was non-storied with axially elongated fusiform initials and ray initials; the ray initials were unicellular, uniseriate with rectangular cells, while the multiseriate rays were comprised of more or less isodiametric cells (Plates- 4.1a, b and 4.3c). The average length and width of fusiform initials ranged from 1148 to 1328 µm and 21 to 37 µm respectively. The ray initials were filled with starch grains and phenolic contents. The fusiform initials showed two to three nuclei and aseptate (Plates- 4.1a, b and 4.3c). The nucleus of each fusiform initial contains one or two nucleoli (Plate- 4.1c).

The initiation of cambial activity was marked by radial swelling of fusiform initials (Plate- 4.2a) and the active vacuolation in the month of May and followed by periclinal divisions in the fusiform initials from May onwards

(Plate- 4.2b). As a result of these periclinal divisions, the numbers of cambial layers increased to four to five layers in May (Plate- 4.2b and Table 4.1). The average width of cambial zone also increased to 64.75 µm in the month of May (Table 4.1). Consequently, the number of cells in the cambial zone and the average width of cambial zone increased considerably. The cambial activity reached its peak in the month of August with eight to nine layers in the cambial zone (Plate- 4.3a) and the average width of cambial zone was 96.25 µm (Table 4.1). During the active period of the vascular cambium in eight months i.e. from the first week of May to the last week of December, the cambial zone was wider consisting of five to nine layers in each radial file (Table 4.1). The cell walls of both the fusiform and the ray initials were thin, and the beaded appearance of fusiform initials was not much prominent in the active period of the vascular cambium (Plate- 4.1a, b). The average length of fusiform initials varied from 1203 to 1328 µm when the vascular cambium was in active period (Table 4.1).

The cambial activity was slowed down from October to the end of December. The cessation of cambial activity began towards the end of December, and dormancy was imposed for four months from January to the end of April (Fig. 4.1). During the dormant period, the cambial zone was narrow, consisting of three to four layers only with relatively thick radial walls in transverse section and surrounded by mature xylem and phloem elements (Plate- 4.3b and 4.5b). The beaded appearance of the cell wall of fusiform initials and ray initials was more prominent in dormant cambium (Plate- 4.3c).

The average length of fusiform initials was maximal during peak cambial activity and was minimal during cambial dormancy. The average data pertaining to numbers of cambial layers and average width of cambial zone for different months was given (Table 4.1 and Fig. 4.2a).

Differentiation of secondary xylem

Xylem production was noticed for a total period of about eight months in *D. indica*, with the formation of new xylem fibres, vessel elements, axial parenchyma and xylem rays in the month of May and it was continued up to the last week of December (Fig. 4.2a and Table 4.1). Maximum xylem production was seen from the months of June to October and the average width of differentiating xylem zone ranged from 81 to 122 µm (Table 4.1). The xylem fibres produced during May were thin walled with larger lumen and angled in transverse section, the cell wall thickness ranged from one and half to two µm (Plate- 4.3a), in contrast, cell wall thickness of the late wood fibres produced during November ranged from eight to ten µm, and the fibres were radially compressed and the lumen was very narrow (Plate- 4.3b).

Similarly, the vessel elements produced during the active period had scalariform perforation plates with 50 to 60 bars (Plate- 4.4a, b), whereas those produced during dormancy had the scalariform perforation plate with 25 to 30 bars (Plate- 4.4c). During the period of xylem differentiation, the average length of xylem fibres and vessel elements ranged from 1731 to 1868 µm and 1110 to 1182 µm respectively (Table 4.1). Details regarding the timing of initiation and the cessation of xylem production as well as its duration and the average width of differentiating xylem zone are given (Table 4.1 and Fig. 4.2a).

A comparison was made between the average length of fusiform initials with that of libriform fibres and vessel elements in different months of the year to find out the change in length, if any, during differentiation of xylem fibres and vessel elements. The average length of xylem fibres and vessel elements varied from 1644 to 1868 µm and 1011 to 1182 µm respectively (Table 4.1). There was an increased of 41% in the average length of xylem fibres and decreased of 12% in length of vessel elements when compared with that of the average length of fusiform initials. The average width of xylem fibres ranged from 21 to 39 µm and vessel elements from 46 to 74 µm. The width of xylem fibres and vessel elements showed an increased of 0.34% and 10% respectively from that of fusiform initials. The average length of xylem fibres and vessel elements showed the same trend of variations as that of fusiform initials in *D. indica* (Fig. 4.2 b).

Structure of secondary xylem

The secondary xylem of *D. indica* consists of libriform fibres, vessel elements and axial parenchyma apotracheal diffuse and paratracheal vasicentric scanty. The wood was diffuse porous (Plate- 4.5a) and the growth rings were scarcely distinct, delimited by smaller vessel and denser fibrous tissue in the outer portion of the ring. The fibres were coarse, angled in the transverse section and more or less aligned in radial row (Plates- 4.3b and 4.5a); the last several rows in growth ring are usually with greater shortened radial diameter, frequently contiguous to the vessel. The vessel elements were generally arranged in solitary (Plate- 4.5a). The end wall of vessel elements showed scalariform perforation plate and the lateral wall pittings was scalariform type (Plate- 4.4a, b, c).

Starch grains, polyphenol and tannin contents are the major reserved products found in the ray initials of cambium, xylem rays, and axial parenchyma cells and occasionally in the xylem fibres during dormancy (Plates- 4.5b and 4.6a). The starch grains and protein bodies were masked by abundant phenolic contents in the ray initials. The raphids bundles were noticed in the unicellular ray initial and on the phloem side (Plates- 4.5b and 4.6a); on the other hand, starch grains were more in the xylem rays and xylem parenchyma cells. During the onset of cambial activity, the amount of starch grains was reduced but not totally absent. Phenolic substances do exist in both dormant and active periods of the vascular cambium (Plates- 4.1a, b, and 4.3a).

Cambial activity in relation to phenology

The first visible indication of dormancy breakage in *D. indica* was a slight swelling of young vegetative buds in the middle of April. Reactivation was marked by radial swelling of the fusiform initials and active vacoulation in the first week of May i.e. 10 to 15 days after the formation of new leaves and buds (Plate- 4. 2a). This was followed by few periclinal divisions (Plates- 4. 2b and 4. 6 b, c) from the month of May onwards and as a result, the cambial layers increase to·four to five layers in May. By the middle of May, the maximum leaf flushing started and continued up to the end of December. Sprouting of new leaves as well as persistent mature leaves was observed from middle of April to January. The number of cambial layers ranged from five to nine in the months of July and August. Consequently, the cambial zone width also increased (Table 1). Leaf senescence was initiated from the month of February, and the defoliation continued up to the end of March. The tree was completely barren for a brief period of 15 days; therefore, *D. indica* belongs to brevi-deciduous type (Borchert 1999). During winter, the vascular cambium consisted of only three to four layers and the average width of the cambial zone remained more or less the same and it varied from 41 to 49 µm (Plates- 4. 3b and 4.5b). There was a correlation between leaf fall and cambial dormancy (Fig. 4.1). Flowering was noticed in the months of June and July and the cambial layer varied from six to eight. Fruiting was observed in the month of August, and mature fruits were borne on tree from September to the last week of October and during this period the number of cambial layers was found to varied from six to nine. The dispersal of seeds took place in the months of October to December (Fig. 4.1).

Relationship between climatic factors, cambial activity and xylem production

The different climatic factors during the study period of the years 2002-2003 are given (Fig. 4.3; detailed in Materials and Methods). The cambial parameters (the average width of cambial zone, the average width of differentiating xylem zone, the average length of fusiform initials, xylem fibres and vessel elements) showed significant (*r*) values with all the climatic factors (monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity) (Table 4.2 and Figs. 4.4 a-o). All these relationships were high explaining more than 40% of the variability between two variables. During the onset of cambial reactivation and the differentiation of xylem elements from the first week of May to the last week of December, a strong positive correlation was shown with monthly minimum ambient temperature and the correlation coefficients were very high (Table 4.2). The response function and the correlation coefficient of the cambial activity (includes average width of cambial zone, average length of fusiform initials) versus monthly mean minimum temperature ($r= 0.85$; $r = 0.90$) was higher than the monthly mean temperature and mean maximum temperature respectively (Table 4.2). Similarly, the width of differentiating xylem zone, average length of xylem fibres and vessel elements showed more positive correlation coefficient with the ambient monthly mean minimum temperature ($r = 0.88$; $r = 0.89$ and $r = 0.82$) than precipitation and relative humidity (Table 4.2).

Therefore, multiple (partial) regression analysis was carried out to see how much every climatic parameter contributes to the variability of the activity of the vascular cambium. The multiple (partial) regression analysis has shown that monthly mean minimum temperature was statistically significant with the average width of cambial zone ($t \geq +2.99$), the average length of vessel elements ($t \geq +2.45$) and the average width of differentiating xylem zone ($t \geq +2.64$). However, monthly rainfall was statistically significant but had inverse relationship with the average width of cambial zone ($t \geq -2.92$), average length of fusiform initials ($t \geq -2.50$), average length of vessel elements ($t \geq -2.29$) and the average width of differentiating xylem zone ($t \geq -2.55$) (Table 4.2). The length of xylem fibres did not show any relationship with any one of the climatic parameters because its t-value is less than 2.26. Other climatic parameters were not statistically significant with any one of the quantitative data of the vascular cambium and its derivatives. However, among the mean maximum, mean minimum and mean temperature, the B-value of the mean minimum temperature showed higher value than the rest. Therefore, the mean minimum temperature had some effect on the cambial activity and wood formation. Moreover, during the onset of the cambial reactivation and differentiation of xylem elements during first week of May to the last week of December, a positive correlation was shown with monthly minimum ambient air temperature and the correlation coefficients are very high (Table 4.2 and Figs. 4.4 a-o). The effect of rainfall and relative humidity on both the cambial activity and differentiations of xylem elements was secondary in nature.

Fig. 4.1 Phenology, duration of cambial activity and dormancy in *Dillenia indica* Linn.

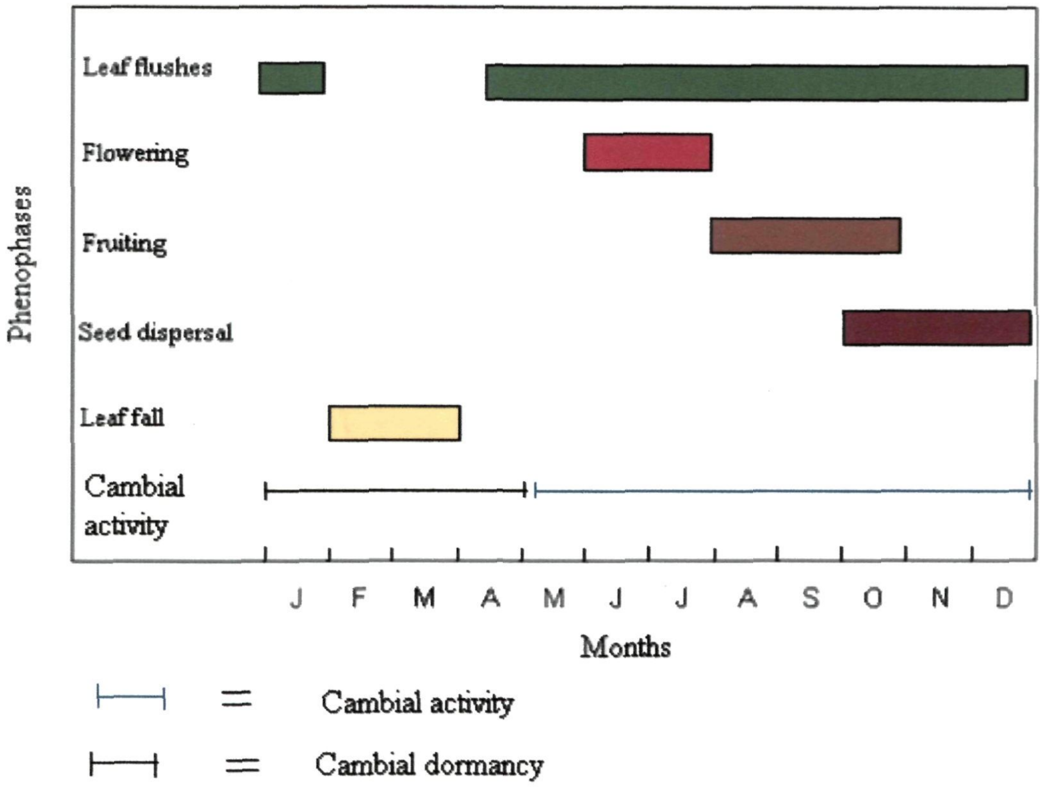


Table 4.1 Average quantitative data of the vascular cambium and its derivatives in *Dillenia indica* Linn.

Months	Numbers of cambial layers	Average width of cambial zone in µm.	Average width of differentiating xylem zone in µm.	Average length of fusiform initials in µm.	Average length of xylem fibres in µm.	Average length of vessel elements in µm.
April	3-4	48.48 ±5.81	–	1215.80 ±27.40	1733.18 ±154.53	1095.41 ± 42.38
May	4-5	64.75 ±6.82	46.23 ±4.87	1264.62 ±41.94	1810.05 ±126.53	1116.61 ±72.42
June	6-7	90.09 ±7.02	97.44 ±5.51	1290.70 ±50.2 0	1798.14 ±169.20	1165.00 ±72.32
July	7-8	103.14 ±5.03	121.18 ±7.49	1284.90 ±29.61	1815.24 ±111.78	1172.76 ±55.60
August	8-9	96.25 ±6.15	114.34 ±8.02	1327.40 ±36.92	1867.58 ±125.17	1181.39 ±85.07
September	7-8	93.60 ±6.47	98.23 ±6.37	1283.30 ±59.81	1807.60 ±147.43	1168.41 ±103.91
October	6-7	87.70 ±6.67	81.22 ±5.63	1266.03 ±46.97	1821.15 ±146.56	1150.63 ±88.11
November	5-6	67.77 ±5.34	58.20 ±4.46	1248.10 ±41.93	1726.35 ±134.06	1138.38 ±79.86
December	4-5	54.56 ±6.04	20.65 ±2.28	1203.16 ±42.84	1731.51 ±131.01	1110.93 ±83.20
January	3-4	41.98 ±6.13	–	1168.16 ±32.23	1653.44 ±98.81	1011.30 ±81.82
February	3-4	42.39 ±5.13	–	1148.40 ±33.21	1644.90 ±103.75	1013.72 ±96.18
March	3-4	45.27 ±6.42	–	1197.04 ±39.34	1746.07 ±155.97	1073.39 ±82.60

– = No xylem production
± = Standard deviation

Table 4.2 The correlation coefficients (*r*) and (*t*) values of the vascular cambium and its derivatives versus different climatic factors in *Dillenia indica* Linn.

	Monthly mean temperature in°C		Monthly mean maximum temperature in°C		Monthly mean minimum temperature in°C		Monthly mean precipitation in mm		Monthly mean relative humidity in %	
	r	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Average width of cambial zone in µm	0.81*	-1.09	0.73*	-1.31	0.85*	+2.99*	0.78*	-2.92 *	0.75*	-0.39
Average length of fusiform initials in µm	0.87*	-0.41	0.81*	-1.61	0.90*	+1.61	0.78*	-2.50 *	0.80*	-0.87
Average width of differentiating xylem zone in µm	0.87*	-0.71	0.84*	-1.18	0.88*	+2.64 *	0.72*	-2.55*	0.69*	+0.48
Average length of xylem fibres in µm	0.88*	-1.42	0.85*	-0.21	0.89*	+1.98	0.70*	-2.16	0.70*	-0.94
Average length of vessel elements in µm	0.80*	-1.34	0.74*	-1.35	0.82 *	+2.45 *	0.66*	-2.29*	0.64*	-1.79

All * *r* and **t* values are significant at $p < 0.05$.

Figs. 4.2 Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Dillenia indica* Linn.

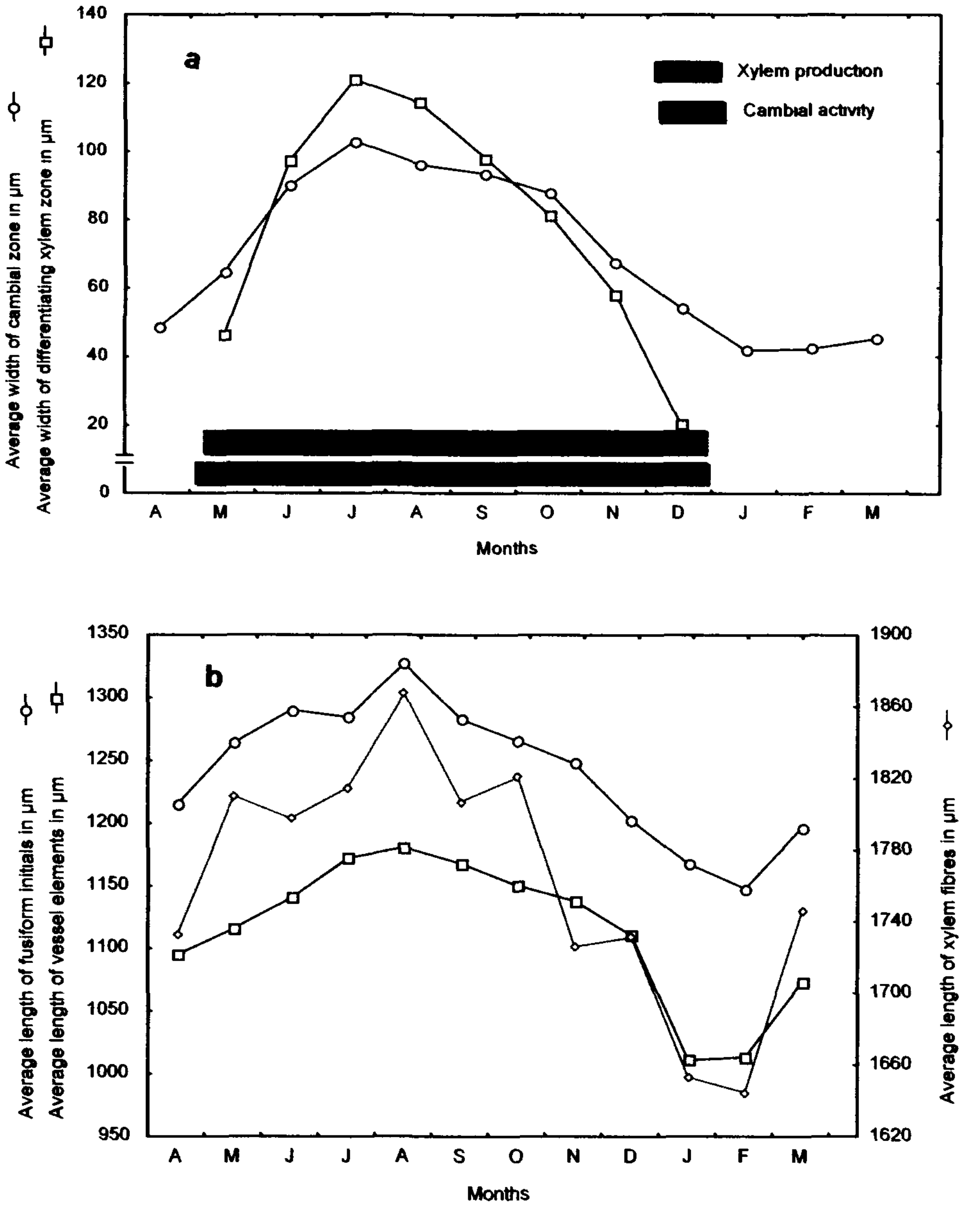
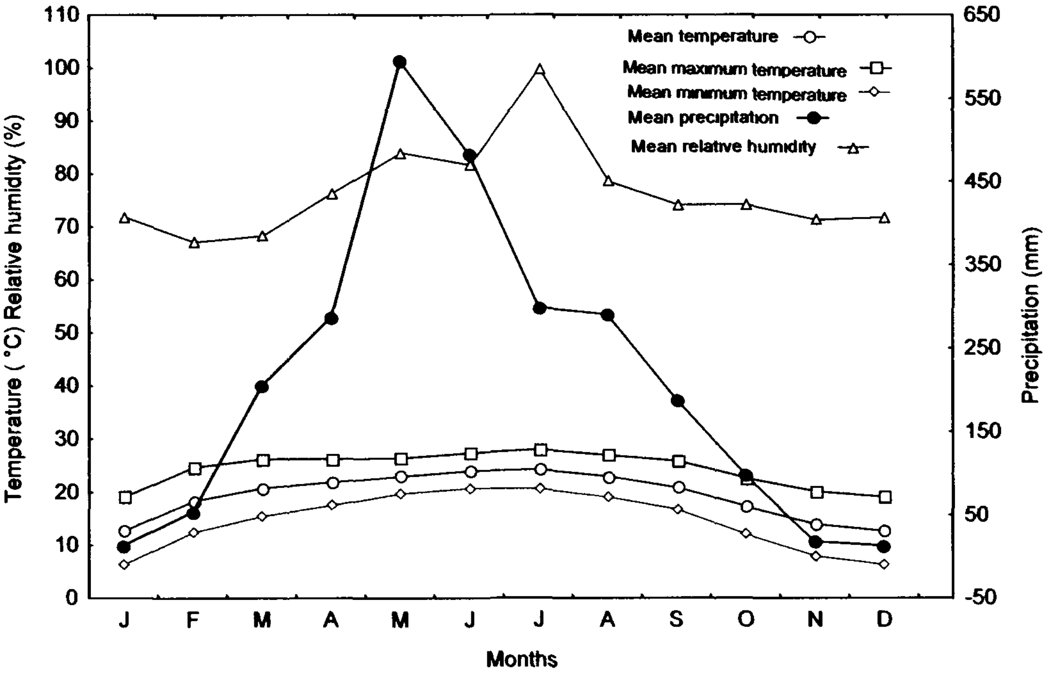
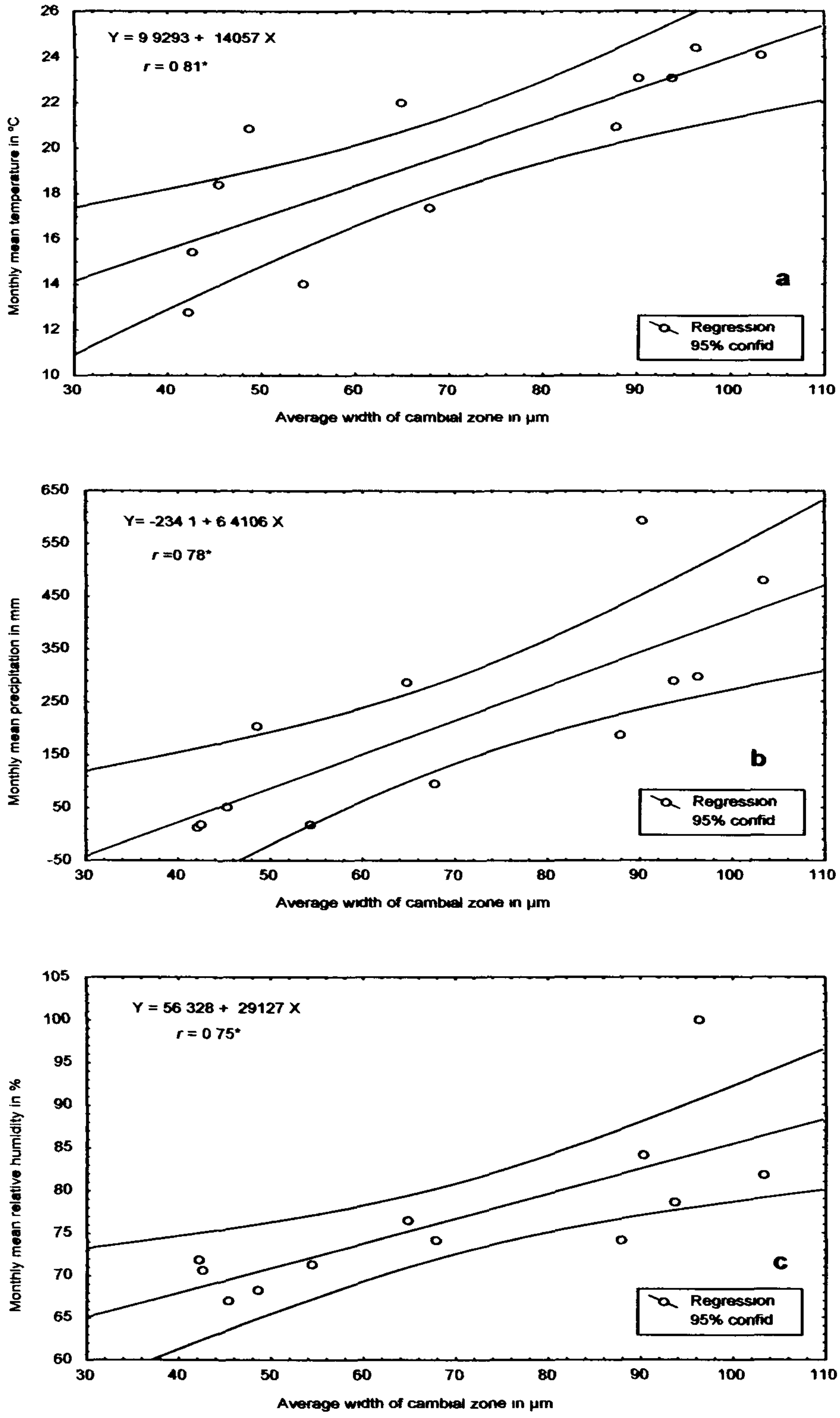


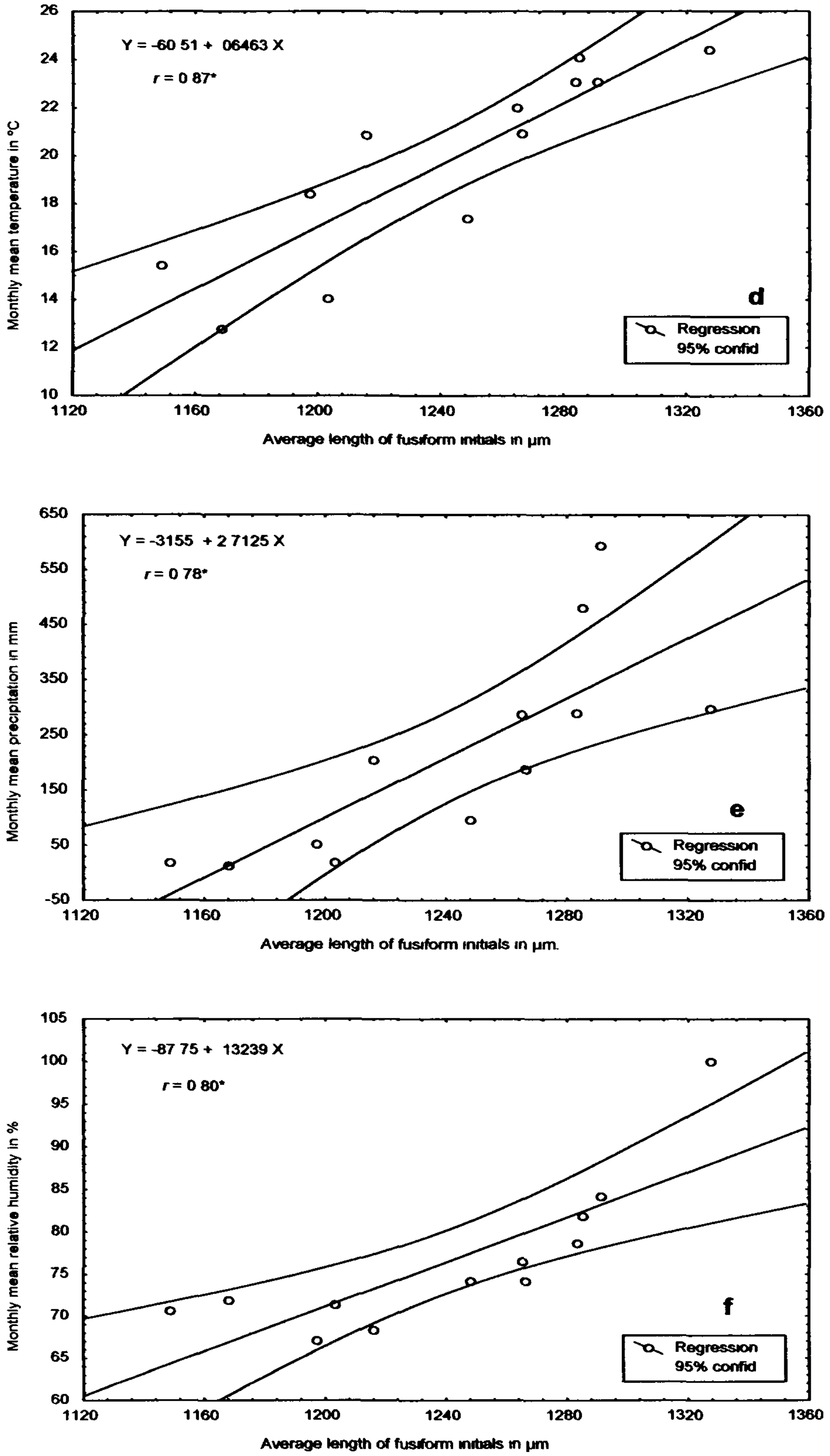
Fig. 4.3 Graphical representation of average climatic data of the years 2002-2003.



Figs. 4.4 Regression line along with regression equation in *Dillenia indica* Linn. All **r* values are significant at $p < 0.05$.



Figs. 4.4 Regression line along with regression equation in ***Dillenia indica*** **Linn. All ****r* **values are significant at** $p < 0.05$.



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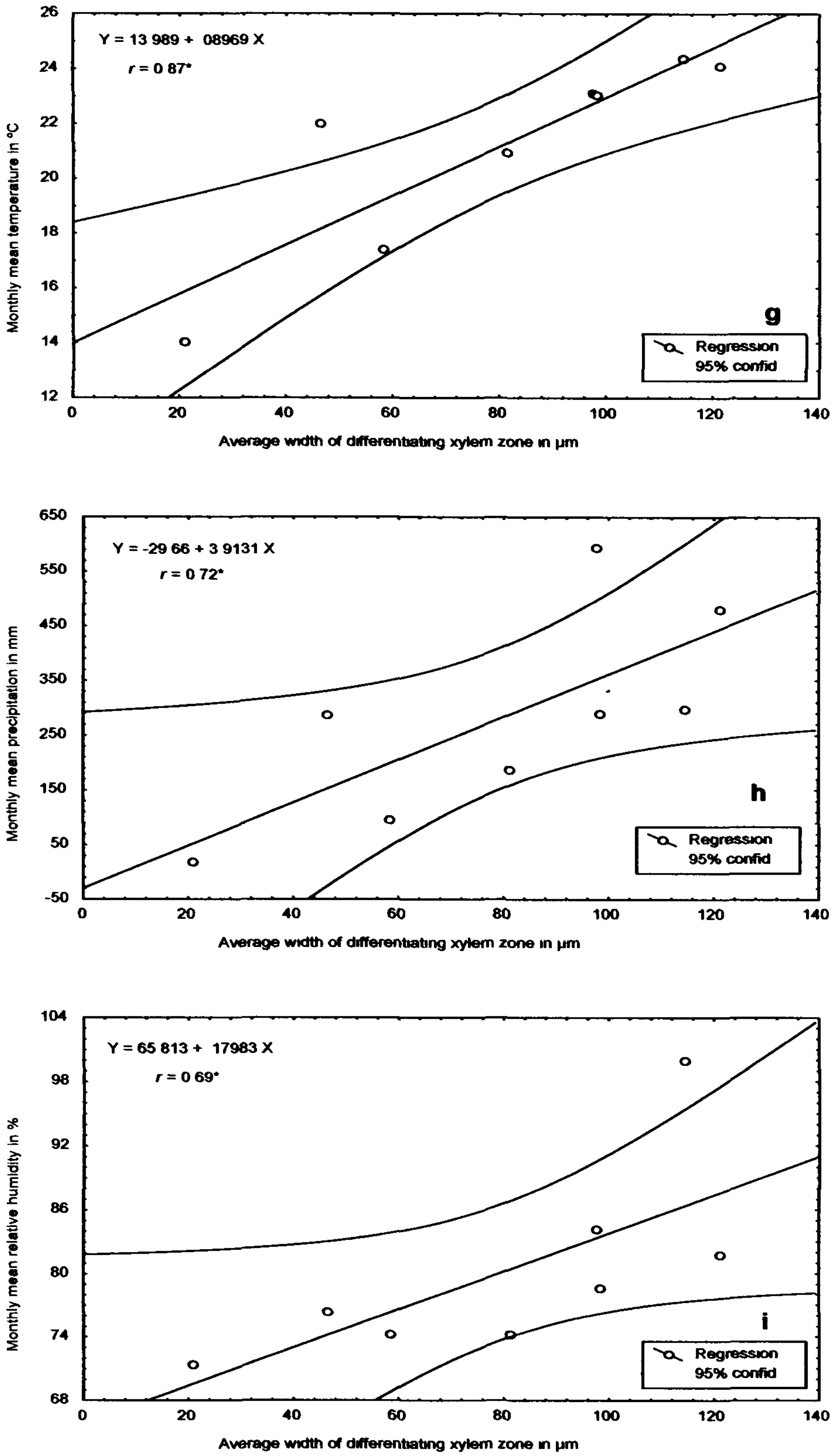
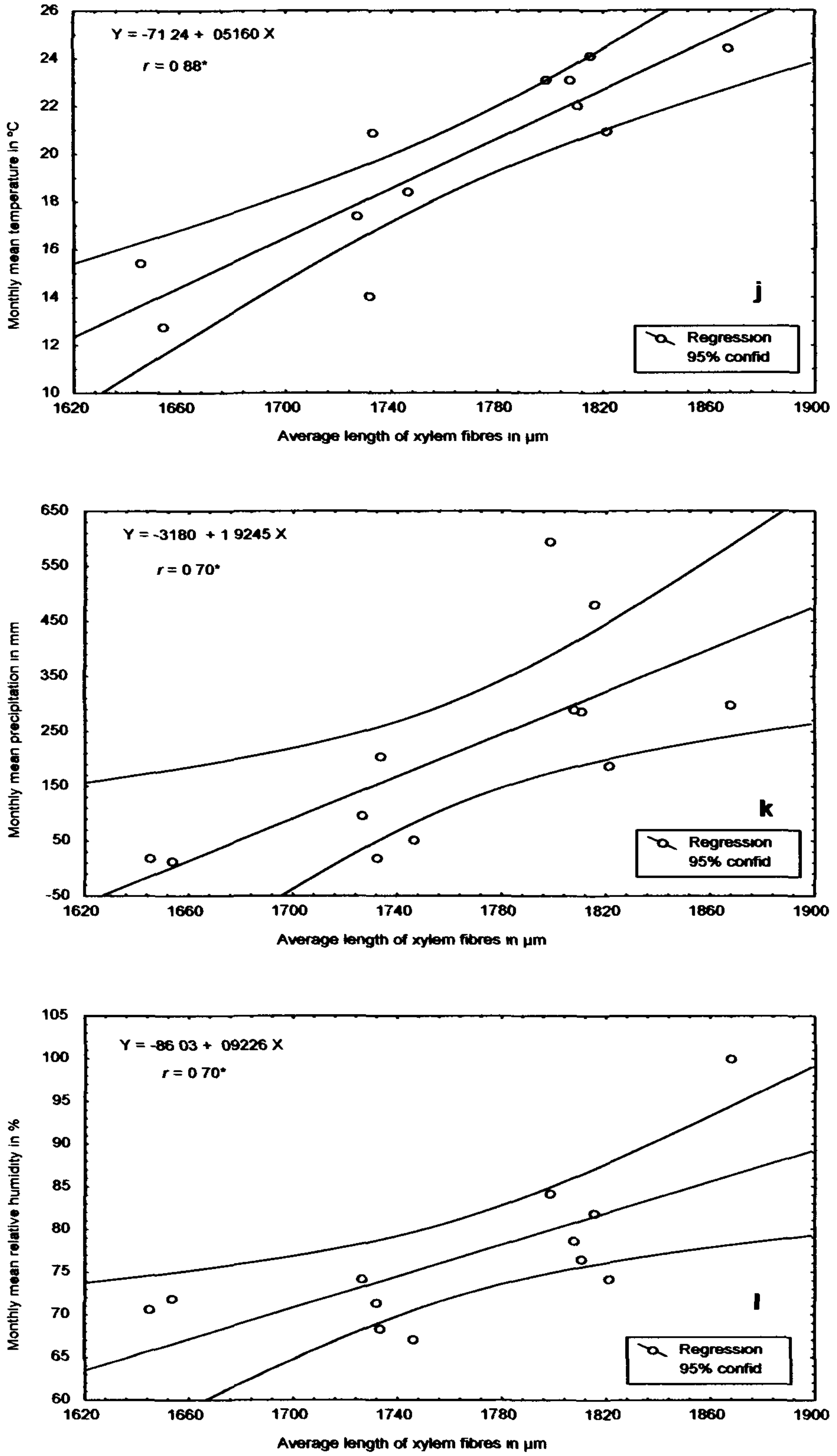


Figs. 4.4 Regression line along with regression equation in *Dillenia indica* Linn. All *r values are significant at $p < 0.05$.



Figs. 4.4 Regression line along with regression equation in *Dillenia indica* Linn. All *r values are significant at $p < 0.05$.

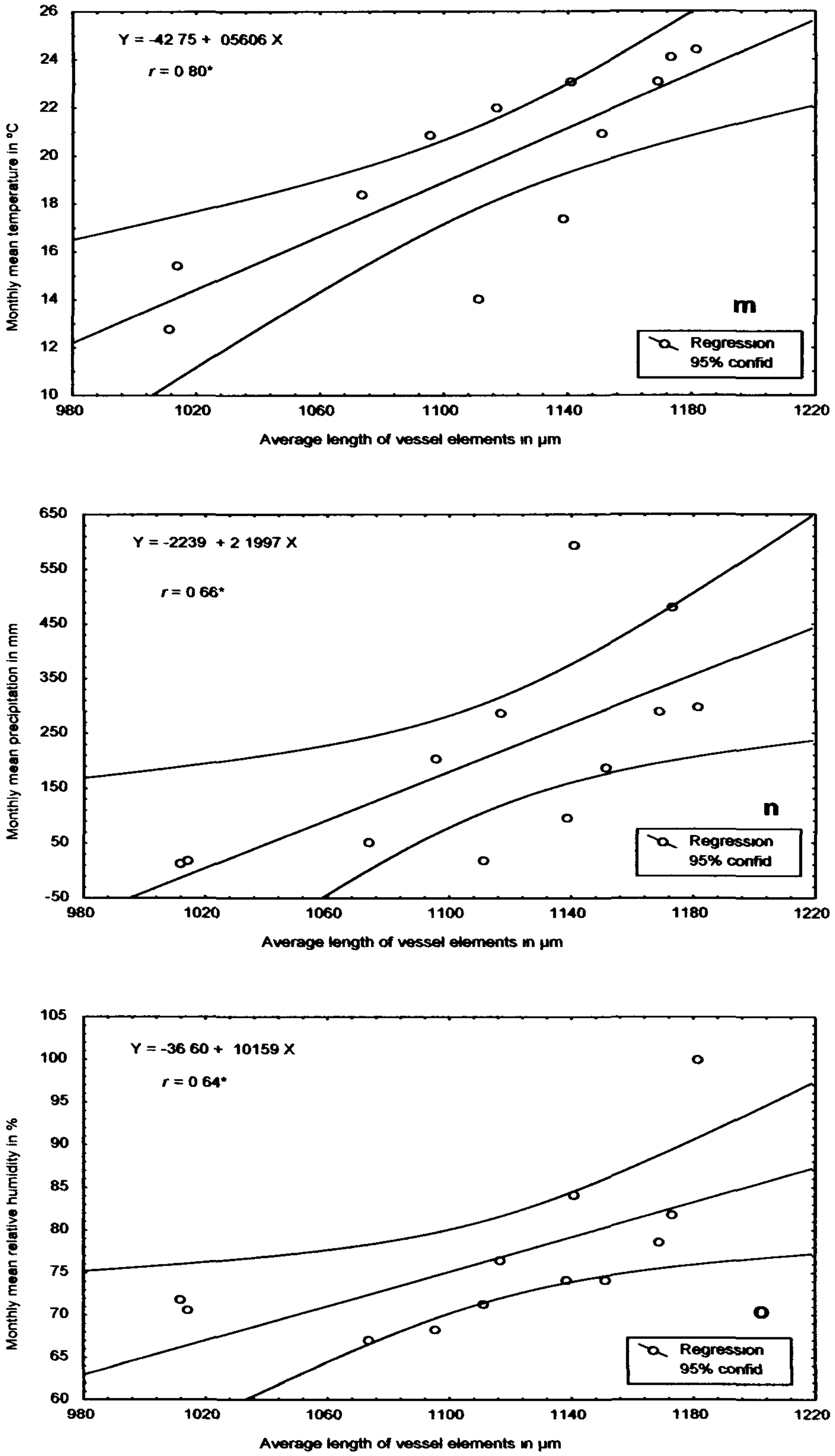


Plate- 4.1

a, b Tangential longitudinal sections (TLS) of active cambium, showing thin cells wall of fusiform initial (Fi) and ray initials (Ri). Beads are not prominent. Note the uniseriate ray (Ur) with rectangular cells and multiseriate ray (Mr) with more or less isodiametric cell with high content of phenols (Pc). Note phragmoplast (white arrow) and daughter nuclei (Dn) in (**b**) during periclinal division.

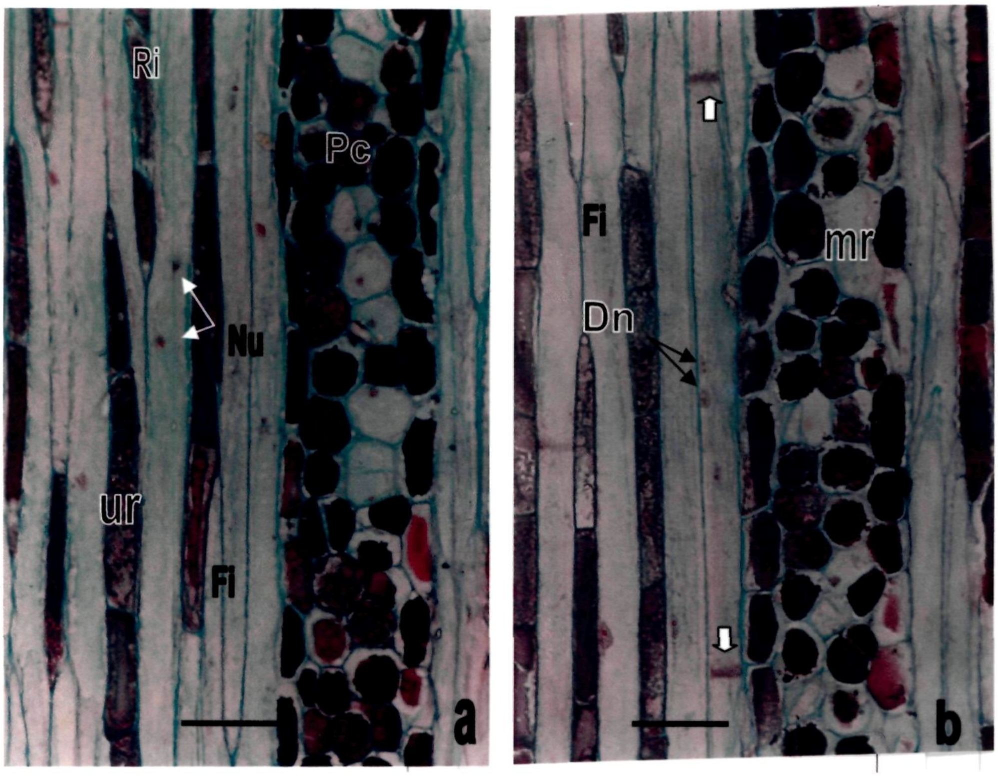
c (RLS) showing two daughter nuclei (Nu) with two nucleoli (Nuc) in each nucleus.

(Bar: **a, b** = 170µm; **c** = 360µm)

Plate- 4.1

Dillenia indica Linn.

Tangential longitudinal sections of active vascular cambium



Two nuclei with 2 to 3 nucleoli

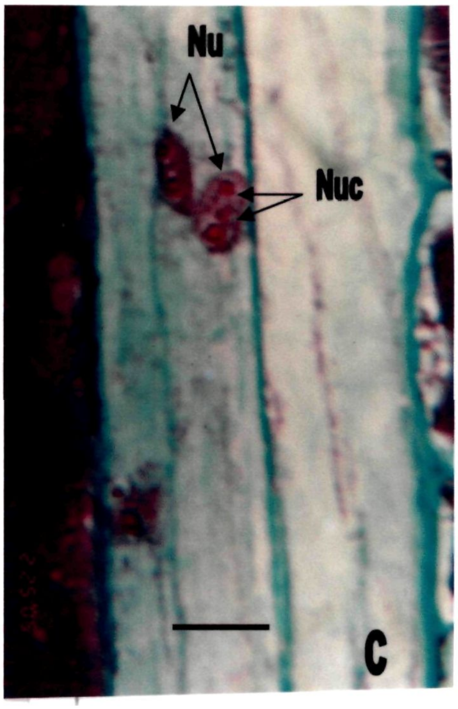


Plate- 4.2

a Transverse sections (TS) at the time of initiation of cambial activity during May. Note the swelling (Rs) of fusiform initials.

b During differentiation, note the periclinal division (Pd) and the cambial zone is demarcatedby white arrows consisting of 4-5 layers.

Double arrows indicate the nuclei.

(Bar: **a** = 15µm; **b** = 20µm)

Plate- 4.2

Dillenia indica Linn.

Cambial Zone

Transverse sections during reactivation

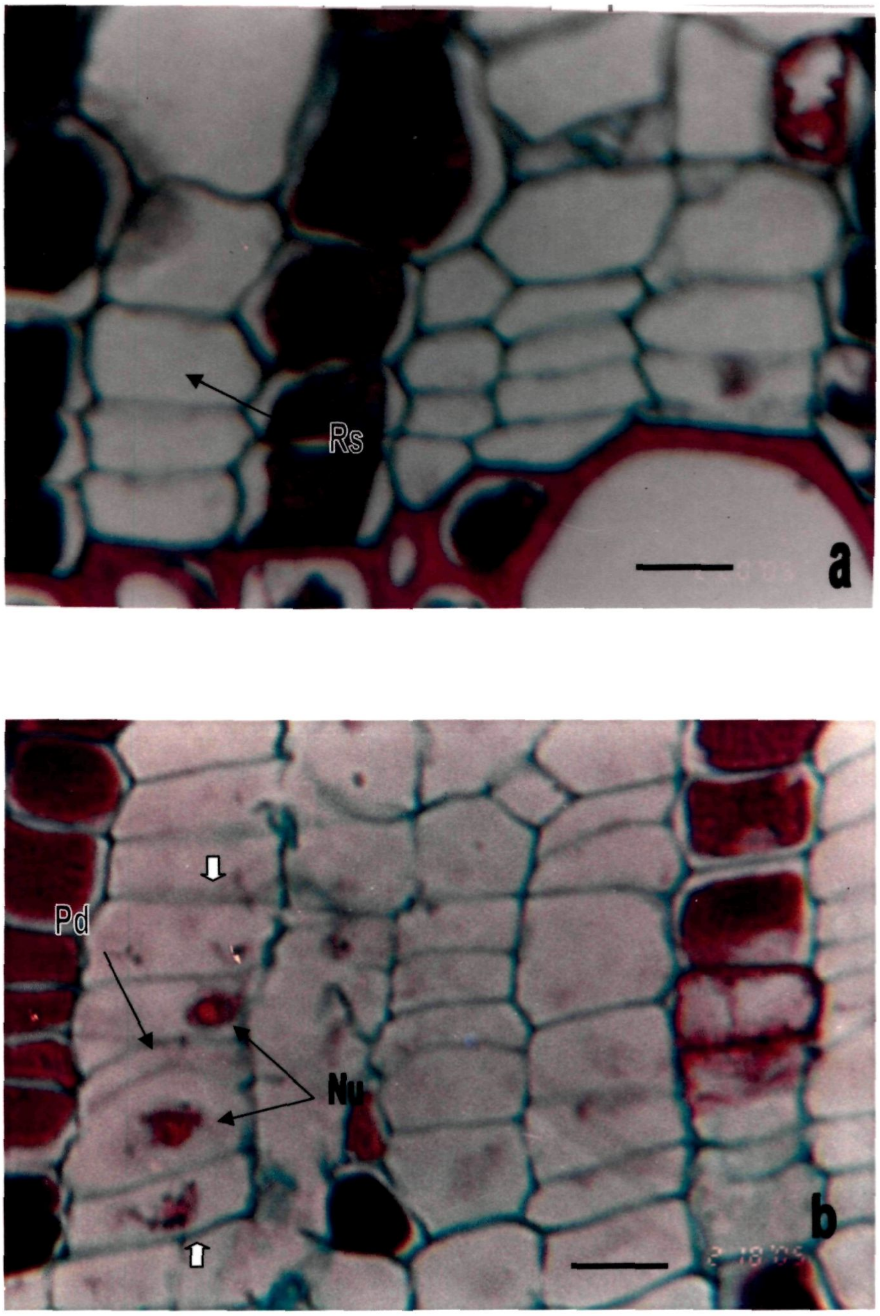


Plate- 4.3

a, b (TS) of active (**a**) and dormant cambium (**b**). Cambial zone (pink arrow head) with 8-9 layers in (**a**), and 3-4 layers in (**b**). Note the differentiating xylem (Dx) and differentiating vessel element (Dv). Phenolic contents (Pc) in (**a** and **b**).

c (TLS) of dormant cambium. The beads are prominent in the radial cell walls of fusiform initials (Fi) and ray initials (Ri).

(Bar: **a** = 70µm; **b** = 60µm; **c** =180µm)

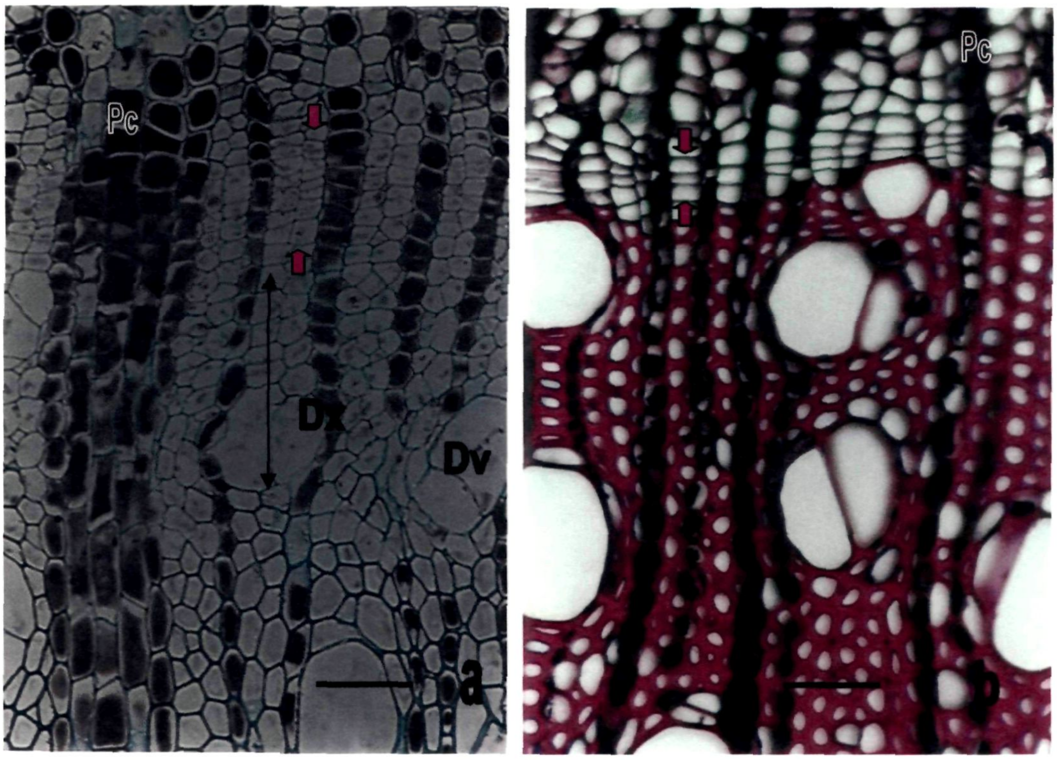
Plate- 4.3

Dillenia indica Linn.

Transverse sections of cambium

Active

Dormant



Tangential longitudinal section of dormant cambium

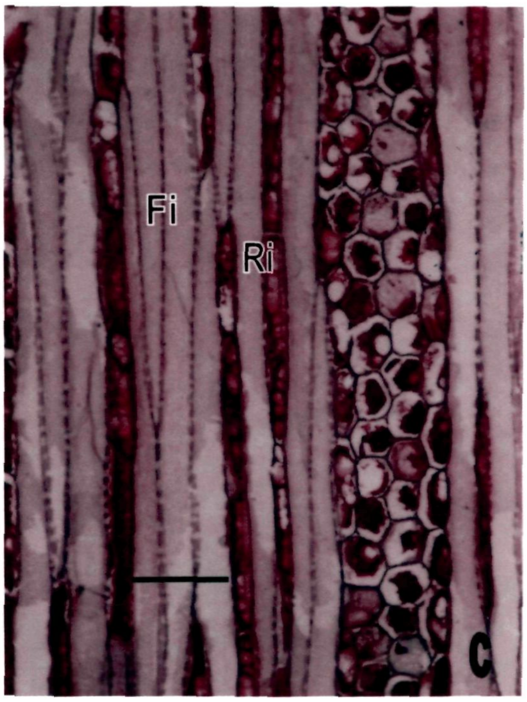


Plate- 4.4

a, b, c Macerated vessel elements. The end walls had scalariform perforation plate (Sp) with 50-60 bars during active period of xylem differentiation in (**a** and **b**) and in (**c**) 25-30 bars in the late wood vessel elements.

(Bar: **a** and **b** = 95µm; **c** = 80µm)

Plate- 4.4

Dillenia indica **Linn.**

Macerated vessel elements

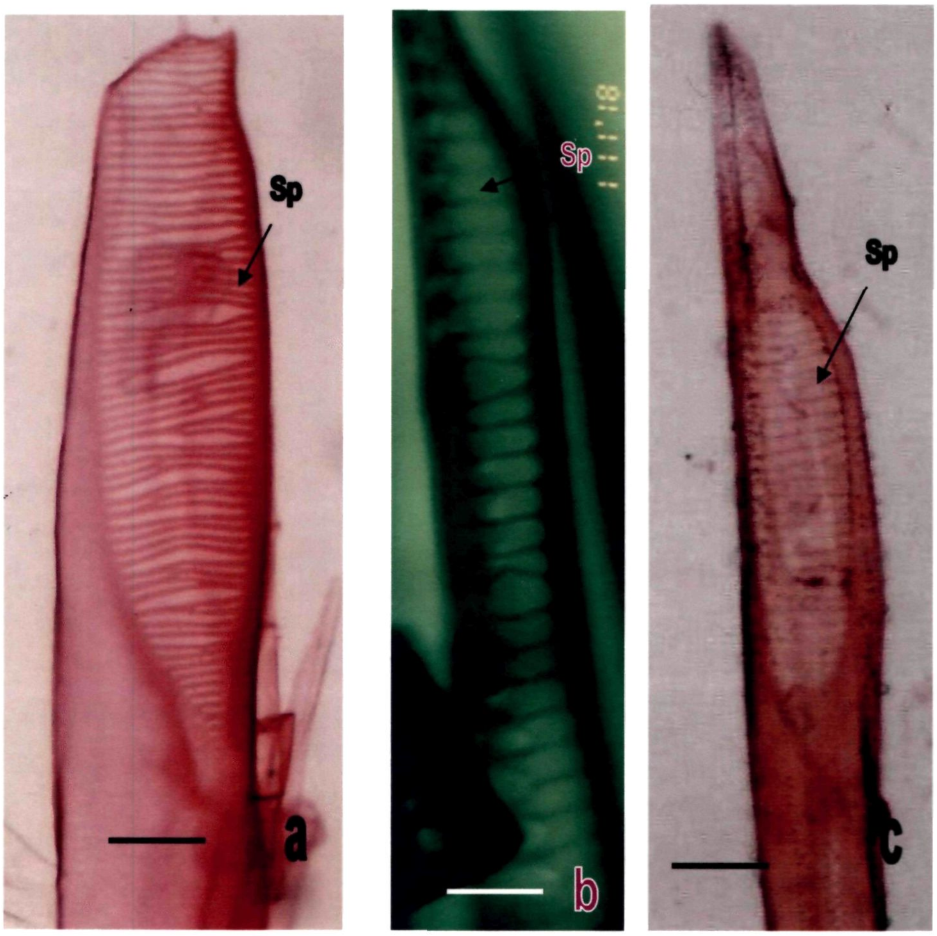


Plate- 4.5

a (TS) of wood (a) with starch grain (S), phenolic contents (Pc) xylem fibres (Xf), vessel elements (Ve), early wood (Ew) and late wood (Lw). Growth ring is indistinct (white arrow head).

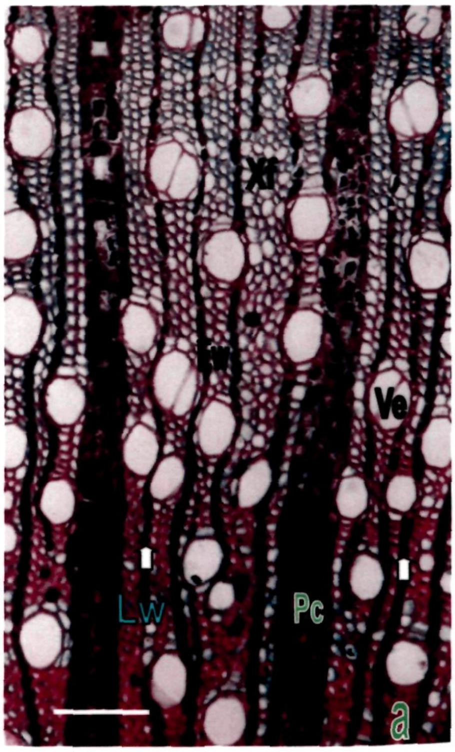
b (TS) of dormant cambium showing starch grain (S), raphide (R) mostly in the phloem side and phenolic contents (Pc) in xylem and phloem rays.

(Bar: **a** = 40µm; **b** = 55µm)

Plate- 4.5

Dillenia indica Linn.

Transverse section of wood



Transverse section of dormant cambium

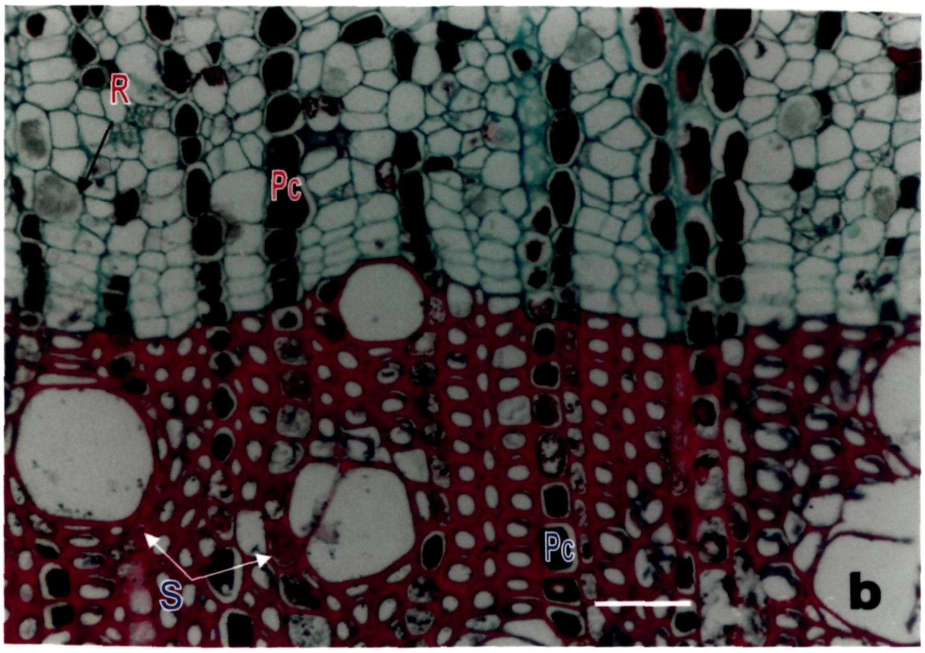


Plate- 4.6

a (TLS) of dormant vascular cambium. Raphide (R) is present in the unicellular ray.

b Periclinal division (Pd) and daughter nuclei (Dn) in the active fusiform initial.

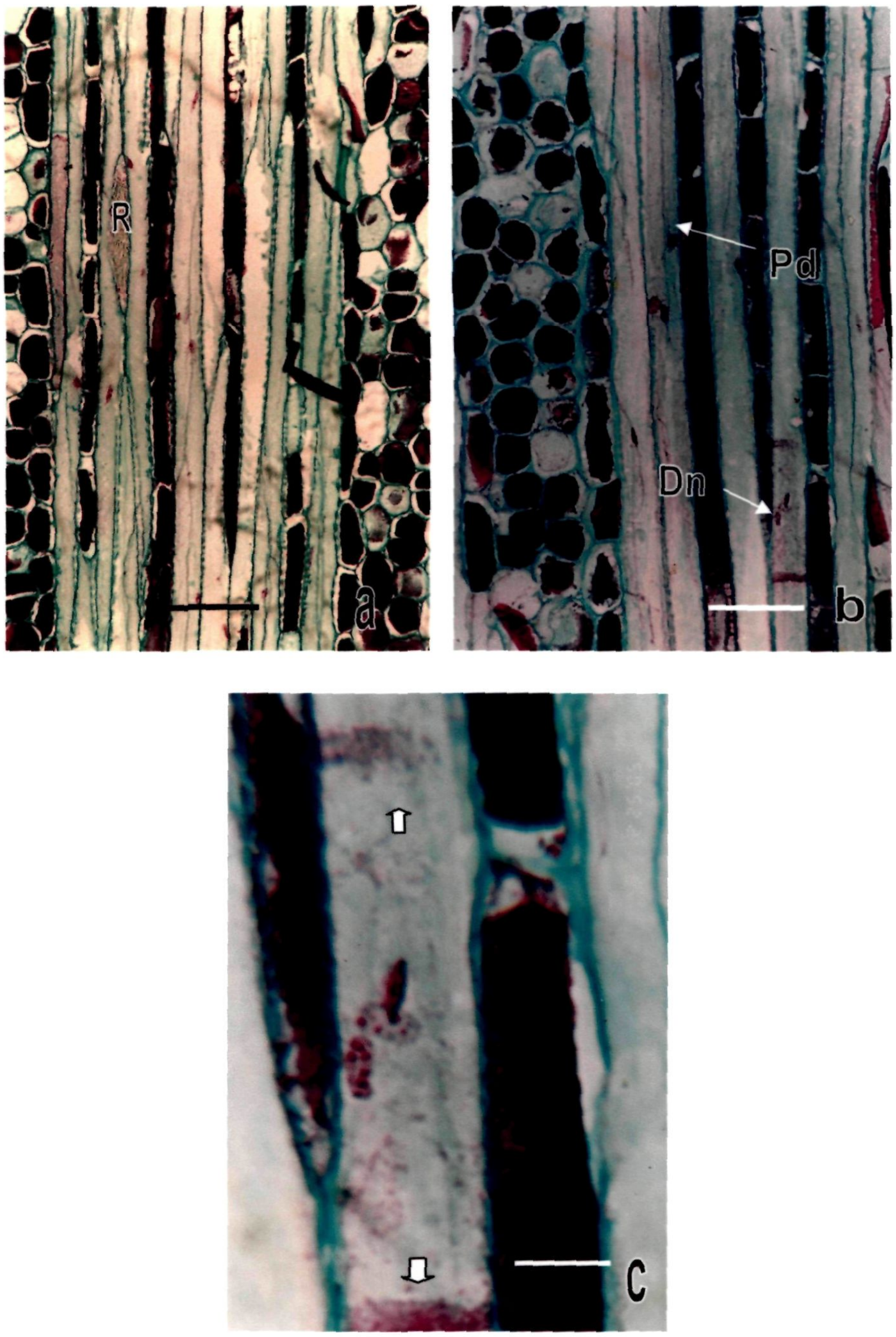
c An enlarged view of (**b**) showing the phragmoplast (white arrows) and two daughter nuclei.

(Bar: **a** and **b** = 180µm; **c** = 390µm)

Plate-4.6

Dillenia indica Linn.

Tangential longitudinal sections of cambium



CHAPTRE - 5

Exbucklandia populnea (R. Br. ex Griffith) R.W. Br.

Introduction

The family Hamamelidaceae, commonly known as "Witch Hazel" family is named after the genus *Hammamelis*. The family Hamamelidaceae bridges the basal elements of the "Rosidae" and the lower "Hamamelidae" (Li et al. 1999). The family consists of about 20 genera and 50 species of evergreen or semi-evergreen trees and shrubs and many species are distributed in Southeast Asia. *Exbucklandia populnea* is an evergreen tree species growing gregariously in the disturbed or secondary, hill to montane rain forest. *E. populnea* is an important timber yielding tree of Meghalaya.

Sharma and Gupta (1988) studied the physical and mechanical properties of wood of *E. populnea* from Kalimpong, West Bengal. The wood anatomy of *Shaniodendron subaequale* (Hamamelidaceae) and its systematic implication was investigated by Fang-Yan-Ming (1996). Carlquist (1988) investigated the wood anatomy of Hamamelidaceae and compiled the different features of wood in this family. Similarly the anatomy of wood Hamamelidaceae was described by Metcalf and Chalk (1950).

Periodicity of the vascular cambium and xylem production is controlled by various environmental and physiological factors (Philipson et al. 1971; Iqbal 1994; Larson 1994; Schrader 2003; Schrader et al. 2004; Grotta et al. 2005; Ko et al. 2006; Druart et al. 2007; Fonti et al. 2007). Seasonal behaviour of the vascular cambium in temperate species has been studied widely in both the gymnosperms and angiosperms than the tropical species (Bailey 1920a; Bannan 1955, 1962; Antonova 1996; Antonova and Stasova 1997; Rensing and Samuel 2004). In temperate climate, cambial activity is a periodic process, which usually occurs during autumn and ceases its activity during winter (Denne and Dodd 1981; Larson 1994; Lachaud et al. 1999; Savidge 2000; Wodzicki 2001; Funada et al. 2002; Larcher 2003; Gricar et al. 2006; Begum et al. 2007).

Studies inferring causal relationship among trees radial growth, phenology and local environmental factors have been carried out in quite a number of plants of temperate and tropical species (Berlage 1931; Evert 1961, 1963b; Paliwal and Prasad 1970; Paliwal et al. 1975; Ghouse and Hashmi 1979; Iqbal 1979; Dave and Rao 1982a, b; Iqbal and Ghouse 1982, 1985b; Ajmal and Iqbal 1987a, b; Venugopal and Krishnamurthy 1987; Priya and Bhat 1999; Ogata et al. 2001; Rajput and Rao 2000a, b; Rao and Rajput 2001a, b; Callado et al. 2004; Schmitt et al. 2004; Akkemik et al. 2006; Heinrich and Banks 2006; Marcati et al. 2006; Yanez-Espinosa et al. 2006).

However, the seasonal cambial activity, differentiation of xylem elements as well as its duration has not been carried out so far in *E. populnea,* growing in sub-tropical wet forest. Therefore, in this chapter, the interrelationship between climatic factors, phenology, seasonal cambial activity and differentiation of xylem elements as well as its duration was examined in detailed in *E. populnea,* growing in sub-tropical moist forest of Meghalaya, India. This study will help to further increasing our knowledge on the tree growth in sub-tropical forest.

Phenology

E. populnea is a medium sized evergreen tree and grow up to 36m tall. The characteristic features of the new leaf emergence in *E. populnea* was evidenced by the formation of broad, coracious, photosynthetic stipules persistent until the new leaf expanded. The young new vegetative buds were formed in the middle of February and the major leaf flushing was observed by the middle of March. The formation of new leaves continued up to the end of November. The leaves were simple and arranged alternately. The leaves of *E. populnea* resemble those of the unrelated *Liriodendron* belonging to the primitive family Magnoliaceae. Being an evergreen tree, *E. populnea* did not shed all the leaves at the same time. However, major defoliation was seen from the month of February to April but it did not cause the tree barren because there was concomitant flushing of new leaves. In other words, complete defoliation did not occurred and the young leaves develop while the older ones was persistent on the tree.

Flowering began in the beginning of May which continued up to the end of July. The flowers were enclosed by a pair of stipules in the young stage, bisexual, borne on peduncle with 7 to 20 flowers as head. Fruiting was observed in the month of July to September and seed dispersal took place in the month of October and November. There were six to eight seeds in each capsule but only the lower 1 or 2 are fertile and the seeds were narrowly winged. Phenological events (leaf flushes, flowering, fruiting, seed dispersal and leaf fall) were given (Fig. 5.1).

Structure and activity of the vascular cambium

In *E. populnea,* the vascular cambium was non-storied with vertically aligned elongated fusiform initials and horizontally oriented ray initials. The fusiform initials had two to three nuclei which were oval or elongated in shape (Plate- 5.1a, b). The nucleus of fusiform initials contained one or two nucleoli (Plate- 5.1c). The average length and width of fusiform initials ranged from 1447 to 1610 µm and 24 to 41 µm respectively. The ray initials were predominantly uni-seriate but occasionally one or two cell in the uniseriate ray initials underwent vertical division to form bicellular within it (Plate- 5.1a). The ray initials invariably contained one or two nucleus which were globular in shape (Plate- 5.1a, b). The ray initials were filled with crystals of calcium oxalate (cystolith) and phenolic contents towards the phloem side during dormancy (Plates- 5.1d and 5.3a). The cytoplasm of fusiform and ray initials were dense during dormancy, in contrast, the cytoplasm was highly vacuolated during active period. The cell walls of both the fusiform and ray initials were thick; the beaded appearances were more prominent in the fusiform initials during the dormant period (Plate- 5.1a). On the other hand, during the active period, cell walls of both the fusiform and ray initials were thin, and the beaded appearance was indistinguishable (Plate- 5.1b).

Reactivation of cambium was marked by the radial swelling and active vacuolation in the fusiform initials in the first week of March. This was followed by a few random periclinal divisions throughout the circumference in the middle of March (Plate- 5.2a). Consequently, the number of cambial layers increased to four to five layers and the average width of cambial zone also increased to 59.22 µm in March (Table 5.1 and Plate- 5.2b). The cambial activity gradually increased and reached its peak period in the month of July in which the cambial zone was consisted of eight to nine layers and the average width of cambial zone was 104.49 µm (Table 5.1 and Plate- 5.2c, d). The vascular cambium showed its activity for nine months from the first week of March to the end of November (Figs.5.1 and 5.2a). The cambial zone was also surrounded by the differentiating xylem and phloem elements in active phase of the vascular cambium (Plate- 5.2c, d). The average length of fusiform initials varied from 1487 to 1610 µm during the active period of the vascular cambium (Table 5.1).

The cambial activity was slowed down from October to November. The dormancy was imposed for three months from December to the end of February. In the dormant period, the cambial zone was narrow, consisting of three to four layers only with relatively thick radial walls in transverse section (Plates- 5.1d and 5.3a). The mature xylem and phloem elements were abutting on both side of the vascular cambium. The average width of cambial zone was more or less consistent and it ranged from 43 to 47 µm during cambial dormancy from December to February. Data pertaining to the variation in numbers of cambial layers and average width of cambial zone were given (Table 5.1 and Fig. 5.2a).

Differentiation of secondary xylem

Xylem production was noticed for nearly eight and half months from middle of March to November end with the formation of new xylem fibres, fibres tracheid, vessel elements, axial parenchyma and xylem rays (Fig. 5.2a). Xylem production was maximum from May to September and the average width of differentiating xylem zone ranged from 119 to 171 µm (Table 5.1). The average length of xylem fibres and vessel elements were ranged from 1670 to 1905 µm and 1370 to 1482 µm respectively during the period of xylem production (Table 5.1). The xylem fibres produced from the middle of March were thin walled with larger lumen and angular in transverse section; the thickness of cell wall ranged from two to four µm in the differentiating xylem fibres (Plate- 5.2c, d). But the cell wall thickness of the late wood xylem fibres produced during October and November was ranged from 10 to 12 µm, and the xylem fibres were radially compressed, rectangular in outline and the lumen was very narrow (Plates- 5.1,d and 5.3a). Similarly, the vessel elements produced during the active period had scalariform perforation plates with 50 to 65 bars (Plate- 5.3b), whereas those produced during October and November had the scalariform perforation plate with 30 to 40 bars only (Plate- 5.3c). Details regarding the average width of differentiating xylem zone, initiation and cessation of xylem production as well as its duration were given (Table 5.1 and Fig. 5.2a).

The length of xylem fibres and vessel elements were compared with the length of fusiform initials in all different months. The average length of xylem fibres and vessel elements ranged from 1588 to 1905 μm and 1309 to 1482 μm respectively (Table 5.1). The average width of xylem fibres including fibre tracheids ranged from 23 to 45 μm and the average width of vessel elements varied from 45 to 76 μm. The increase in average length of xylem fibres over the average length of fusiform initials was 14% but the increase in width of xylem fibres was 0.6% only. The average length of vessel elements become shorter by about 9% and become broader by 8% over the fusiform initials. However, the average width of vessel elements was more than the average width of fusiform initials and xylem fibres. The average length of xylem fibres and vessel elements more or less corresponded with the changes in average length of fusiform initials in all the months during the study period 2002-2003. The seasonal variation in average length of fusiform initials, xylem fibres and vessel elements were given (Table 5.1 and Fig. 5.2b).

Starch grains, phenolic contents and crystal of calcium oxalate (cystolith) are the major reserved products mostly found towards the phloem side but occasionally located in the xylem rays and axial parenchyma during dormancy (Plates- 5.1d, 5.3a). During the peak cambial activity, the starch grains and crystal of calcium oxalate (cystolith) were totally absent.

Structure of secondary xylem

The wood of *E. populnea* was diffuse porous (Plate- 5.4a). Growth rings were indistinct, occasionally indicated by a narrow layer of radially compressed xylem fibres (Plate- 5.4a). The wood is light reddish brown to brown or greyish brown. The heartwood is deep red-brown with a purplish tinge, not clearly differentiated from paler sapwood. The secondary xylem was composed of libriform fibres, fibre tracheids, vessel elements, xylem rays and axial parenchyma. The libriform fibres were, thick walled and non-septate. Nucleated xylem fibres were also present (Plate- 5.4b). The inter fibres pit were common in both radial and tangential walls, orbicular to oval, fibres tracheids wide border and slit-like openings with incipient pores formed by middle lamella which resembled the bordered pits of gymnosperms (Plate- 5.4c, d). Vessel elements were mostly solitary and occasionally in pairs and lie adjacent with one another in tangential plane (Plate- 5.4a). The oblique tapering end walls of vessel elements showed scalariform perforation plates and the lateral wall pittings are also of scalariform type (Plate- 5.3b, c). The vessel-vessel pits were relatively sparse. The vessel elements were angled for the most part in the transverse section. Axial parenchyma was apotracheal diffuse and sparse; rays fine and mostly uniseriate and not always visible to the naked eye.

Cambial activity in relation to phenology

In *E. populnea,* emergence of young vegetative buds within the photosynthetic stipules was seen in the middle of February. A positive correlation exists between cambial reactivation and expansion of new buds and leaves. During the first week of March, i.e. 10 to 15 days after the initiation of new buds and leaves, the fusiform initial showed radial swelling and active vacoulation. This was followed by few periclinal divisions in the middle of March. As a result, the number of cambial layers increased from three to four layers to four to five layers in March. The process of sprouting of new leaves was continuous phenomenon. The maximum leaf flushing was started in the middle of March and it continued up to November though sporadic senescence of older leaves was also seen. Flowering was seen from the month of May to July and during this period the numbers of cambial layers ranged from six to nine. From the months of July to September i.e. during fruiting period, the numbers of cambial layers ranged from six to nine. The numbers of cambial layers during seed dispersal in the month of October and November ranged from four to six. There was a specific period of leaf fall but a gradual senescence and abscission of older leaves were common from February to April and during this period the number of cambial layers ranged from three to six. The cambium was dormant from December to February. In *E. populnea* there was no correlation between leaf fall and cambial dormancy because young leaves emerge in concurrence with the shedding of old leaves. Data on different phenophases (flushes of new leaves, flowering, fruiting, seed dispersal and leaves fall) were given (Fig. 5.1).

Relationship between climatic factors, cambial activity and xylem production

The different climatic factors during the study period of the years 2002-2003 were given in figure 5.3 (see data in Materials and Methods). During the onset of cambial reactivation and the differentiation of xylem elements from March to November, a strong positive correlation was observed with monthly mean ambient temperature. The correlation coefficient (*r*) of the average width of cambial zone (*r*= 0.94) (Fig. 5.4a); average width of differentiating xylem zone (*r*= 0.93) (Fig. 5.4g); average length of fusiform initials (*r*= 0.96) (Fig. 5.4d); average length of xylem fibres (*r*= 0.93) (Fig. 5.4j) and average length of vessel elements (*r*= 0.97) (Fig. 5.4m) with monthly mean temperature were high in *E. populnea*. The correlation coefficient (*r*) and (*t*) values of different anatomical variables such as average width of cambial zone, average width of differentiating xylem zone, average length of fusiform initials, xylem fibres and vessel elements versus different climatic factors such as monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity were given (Table 5.2). The (*r*) values of different climatic factors (monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity) with that of cambial parameter (the average width of cambial zone, the average width of differentiating xylem zone, the average length of fusiform initials, xylem fibres and vessel elements) were above 60 and significant at $p < 0.05$. All these relationships were high explaining more than 40% of the variability between two variables.

Therefore, multiple (partial) regression analysis was carried out to see how much every climatic parameter contributes to the variability of the activity of the vascular cambium. It was shown that monthly mean temperature was statistically significant with the width of cambial zone ($t \geq +2.78$) and width of differentiating xylem zone ($t \geq +3.29$). The response function and the correlation coefficient of the width of cambial zone (*r*= 0.94) (Fig. 5.4a) and width of differentiating xylem zone (*r*= 0.93) (Fig. 5.4g) with monthly mean temperature was also high (Table 5.2). The monthly mean minimum ambient temperature showed statistically significant inverse relationship with the average width of cambial zone (*t* ≥ -2.61) (Table 5.2). However, the average length of fusiform initials, xylem fibres and vessel elements did not show any statistically significant relationship with any one of the climatic parameters because its *t*-value is less than 2.26. The monthly mean rainfall and mean relative humidity did not showed much significant relationship with the cambial parameters.

Fig. 5.1 **Phenology, duration of cambial activity and dormancy in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br.**

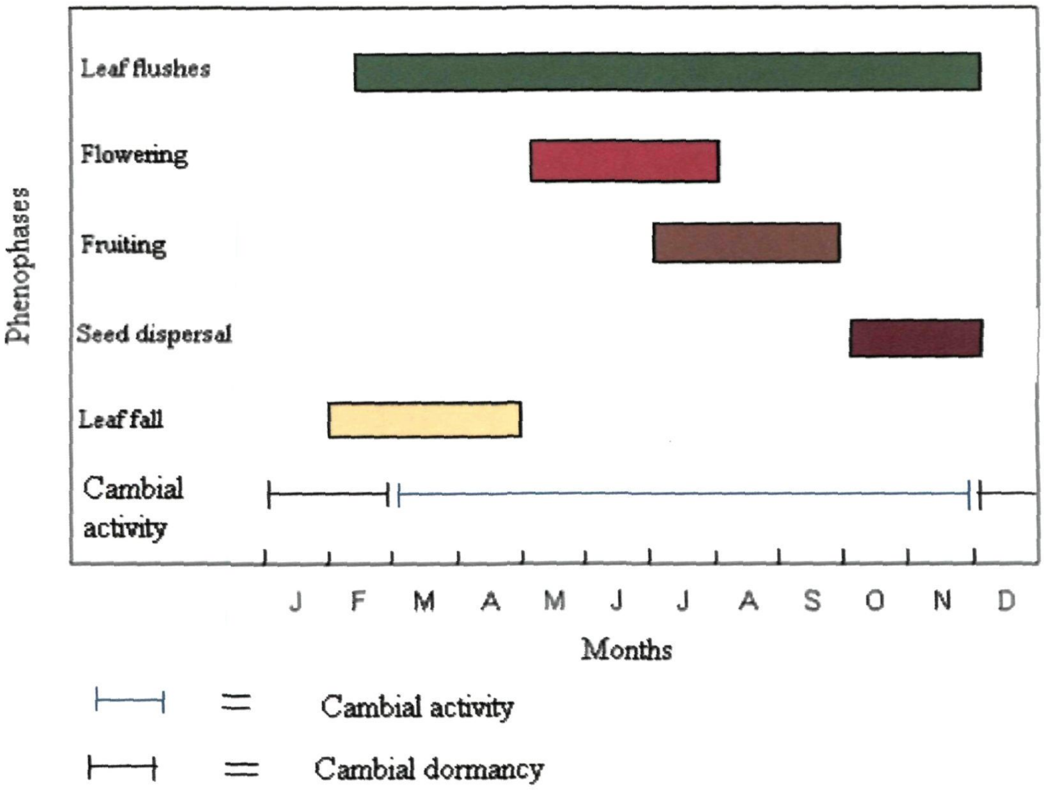


Table 5.1 Average quantitative data of the vascular cambium and its derivatives in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br.

Months	Numbers of cambial layers	Average width of cambial zone in µm.	Average width of differentiating xylem zone in µm.	Average length of fusiform initials in µm.	Average length of xylem fibres in µm.	Average length of vessel elements in µm.
February	3-4	46.57 ± 7.25	–	1451.32 ± 37.80	1609.08 ± 88.21	1350.90 ± 45.10
March	4--5	59.22 ± 8.31	45.06 ± 8.07	1492.71 ± 31.55	1670.45 ± 87.19	1370.45 ± 38.43
April	5-6	73.62 ± 4.84	88.06 ± 9.71	1544.01 ±19.07	1713.63 ± 80.12	1447.72 ± 50.56
May	6-7	87.43 ±11.09	119.29 ±12.60	1584.20 ± 44.31	1820.45 ± 62.06	1443.18 ± 56.03
June	7-8	97.33 ±11.31	158.53 ± 18.18	1601.83 ±15.77	1904.54 ± 51.01	1468.18 ± 50.11
July	8-9	104.49 ± 9.04	170.05 ± 19.74	1609.21 ± 26.23	1881.72 ± 41.96	1452.17 ± 32.50
August	7-8	95.35 ± 7.63	149.62 ± 12.20	1587.14 ± 20.51	1843.18 ± 31.80	1481.82 ± 46.21
September	6-7	86.49 ±11.40	122.40 ±18.93	1596.62 ± 22.89	1900.02 ± 46.09	1461.36 ± 40.87
October	5-6	63.18 ± 6.97	60.03 ± 9.09	1561.53 ± 25.07	1779.54 ± 69.56	1402.27 ± 48.02
November	4-5	54.18 ± 9.05	37.42 ± 6.28	1487.71 ± 32.57	1752.27 ± 41.09	1386.36 ± 34.12
December	3-4	44.37 ± 6.48	–	1447.82 ± 22.82	1593.18 ± 31.80	1320.45 ± 57.89
January	3-4	43.21 ± 5.55	–	1456.31 ± 14.28	1588.63 ± 28.20	1309.08 ± 43.69

– = No xylem production
± = Standard deviation

Table 5.2 The correlation coefficients (*r*) and (*t*) values of the vascular cambium and its derivatives versus different climatic factors in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br.

	Monthly mean temperature in°C		Monthly mean maximum temperature in°C		Monthly mean minimum temperature in°C		Monthly mean precipitation in mm		Monthly mean relative humidity in %	
	r	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Average width of cambial zone in µm	0 94*	+2 78*	0 89*	-1 96	0 95*	-2 61*	0 92*	+2 15	0 69*	+0 61
Average length of fusiform initials in µm	0 96*	+0 31	0 92*	-0 70	0 97*	-0 66	0 89*	+0 14	0 62*	-1 06
Average width of differentiating xylem zone in µm	0 93*	+3 29*	0 83*	-2 01	0 94*	-2 09	0 90*	+2 16	0 70*	+0 74
Average length of xylem fibres in µm	0 93*	+0 92	0 86*	-1 91	0 95*	-0 57	0 89*	-0 91	0 65*	-1 59
Average length of vessel elements in µm	0 97*	+2 11	0 95*	-1 34	0 96*	-2 10	0 83*	+0 50	0 65*	+0 31

All *r and *t values are significant at $p < 0.05$.

Figs. 5.2 Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br.

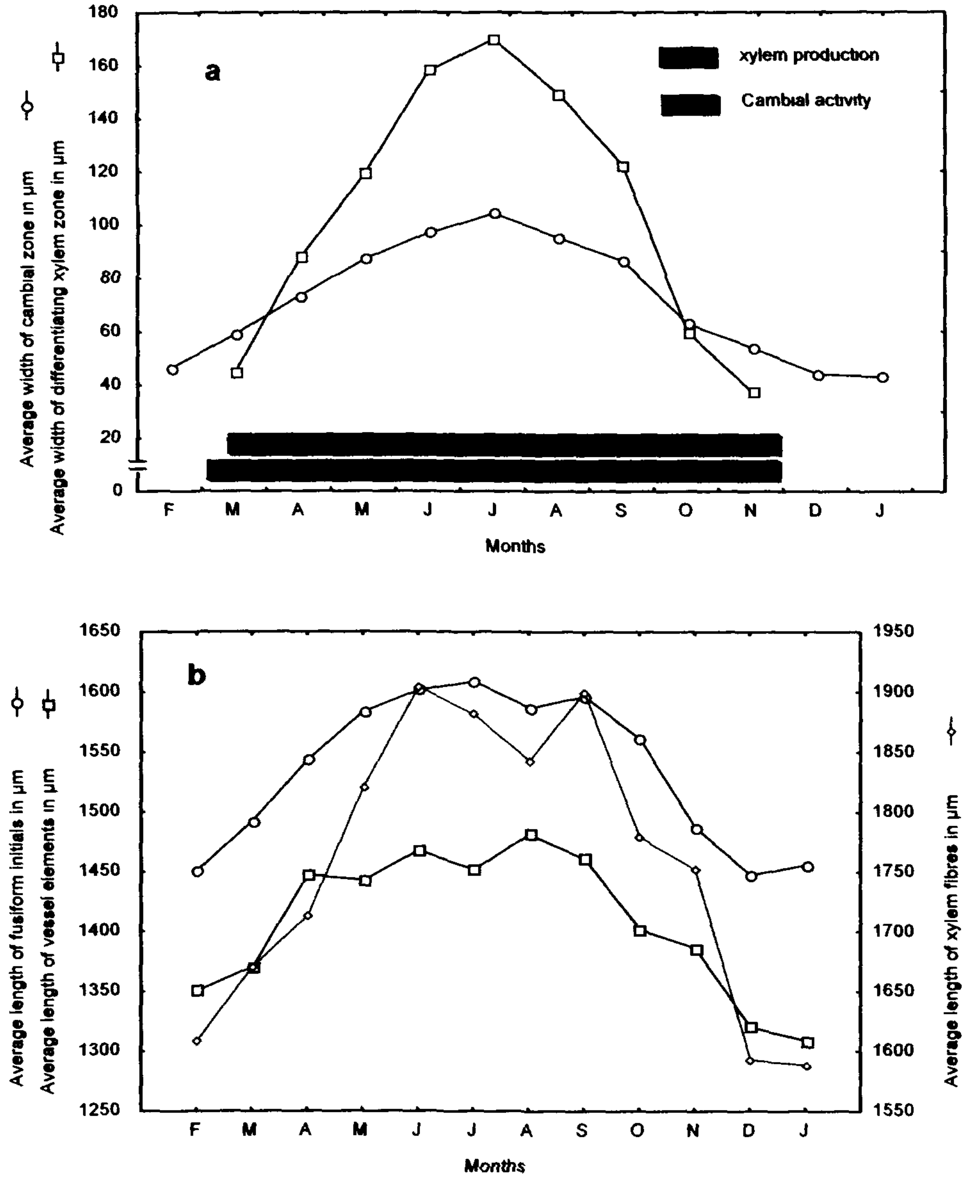
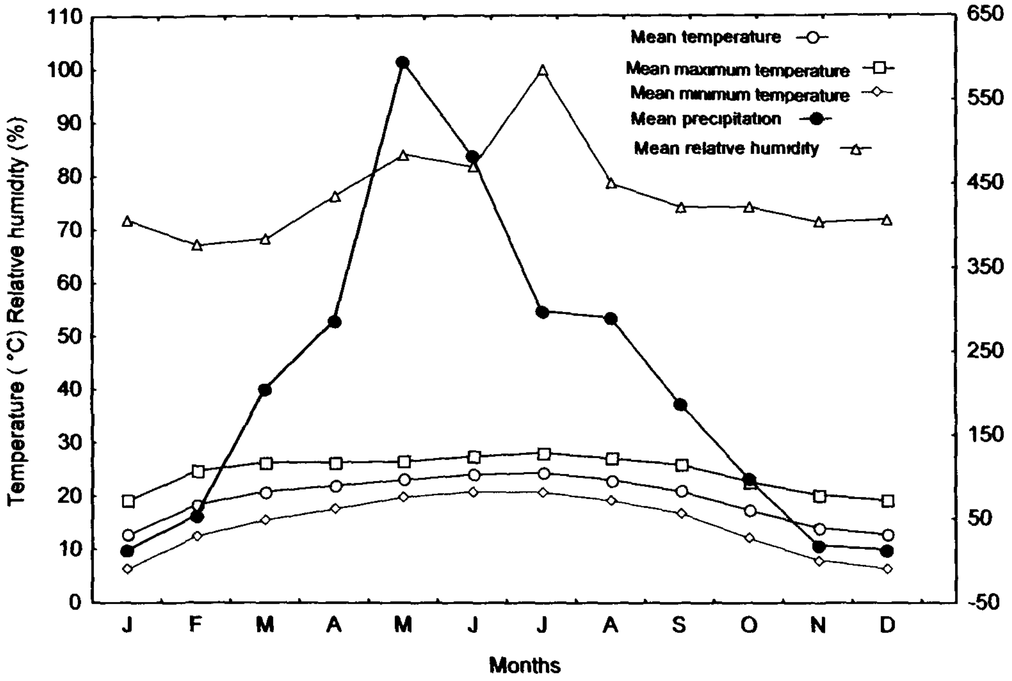
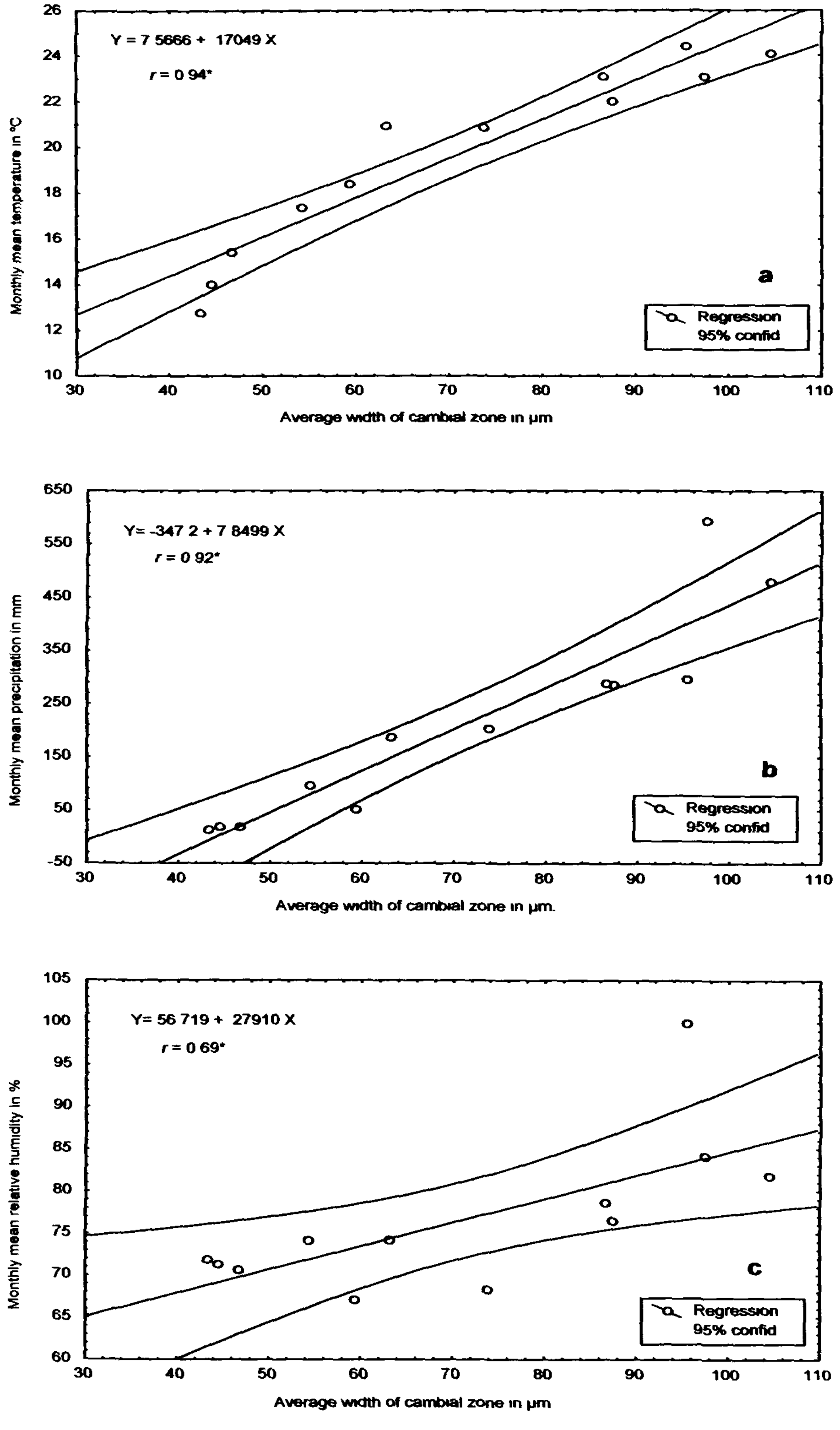


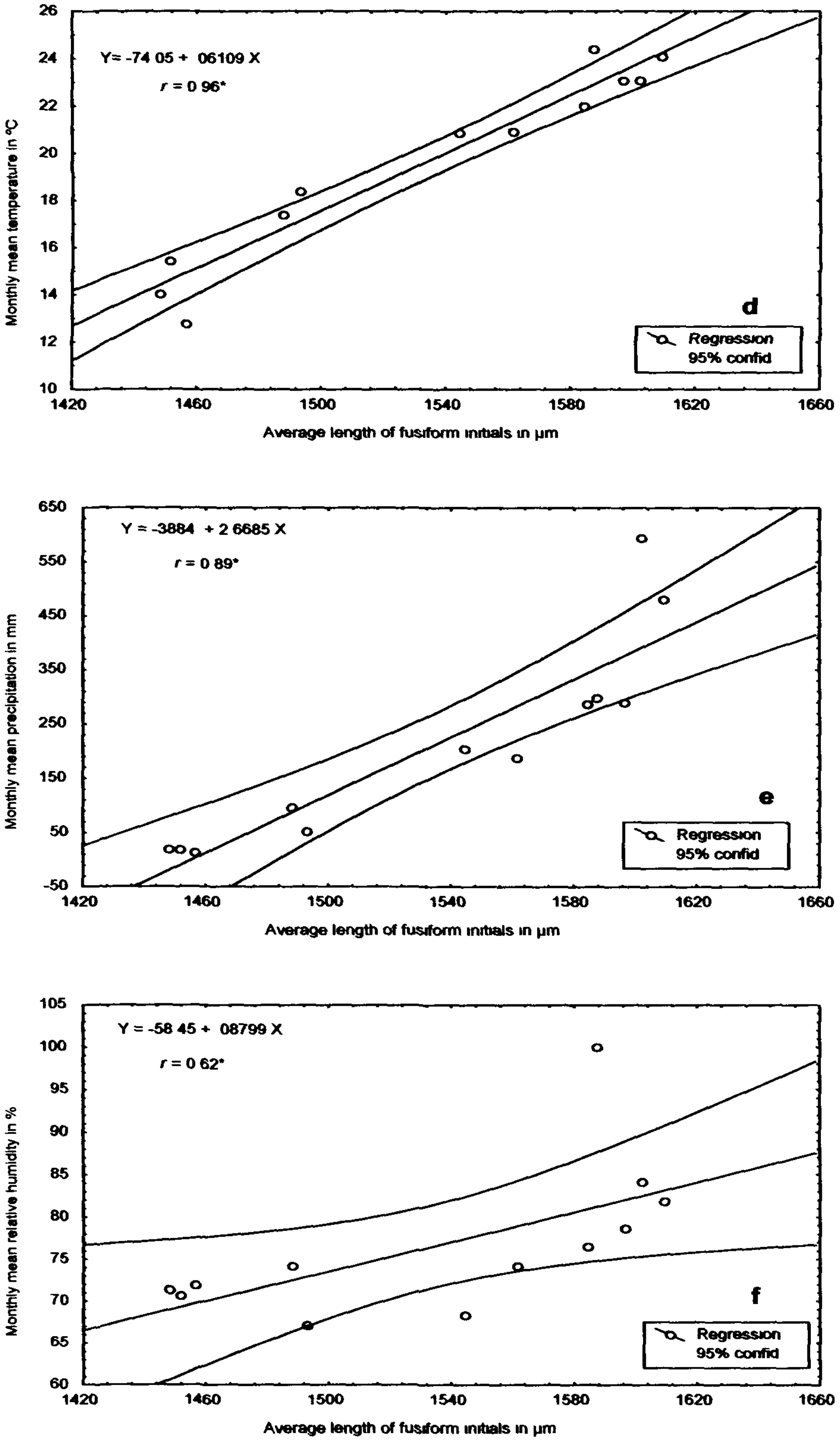
Fig. 5.3 Graphical representation of average climatic data of the years 2002-2003.



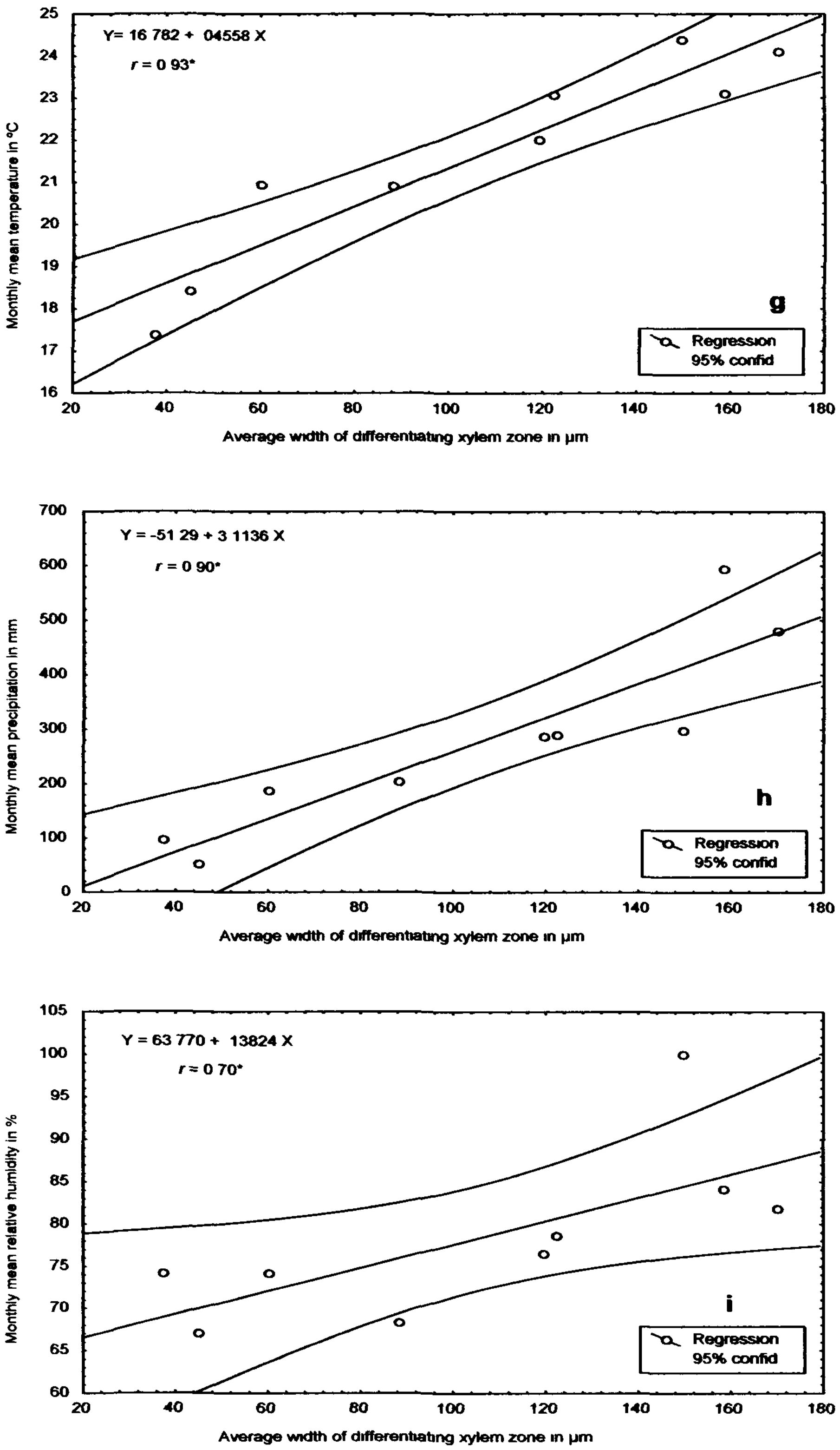
Figs. 5.4 Regression line along with regression equation in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br. All *r values are significant at $p < 0.05$.



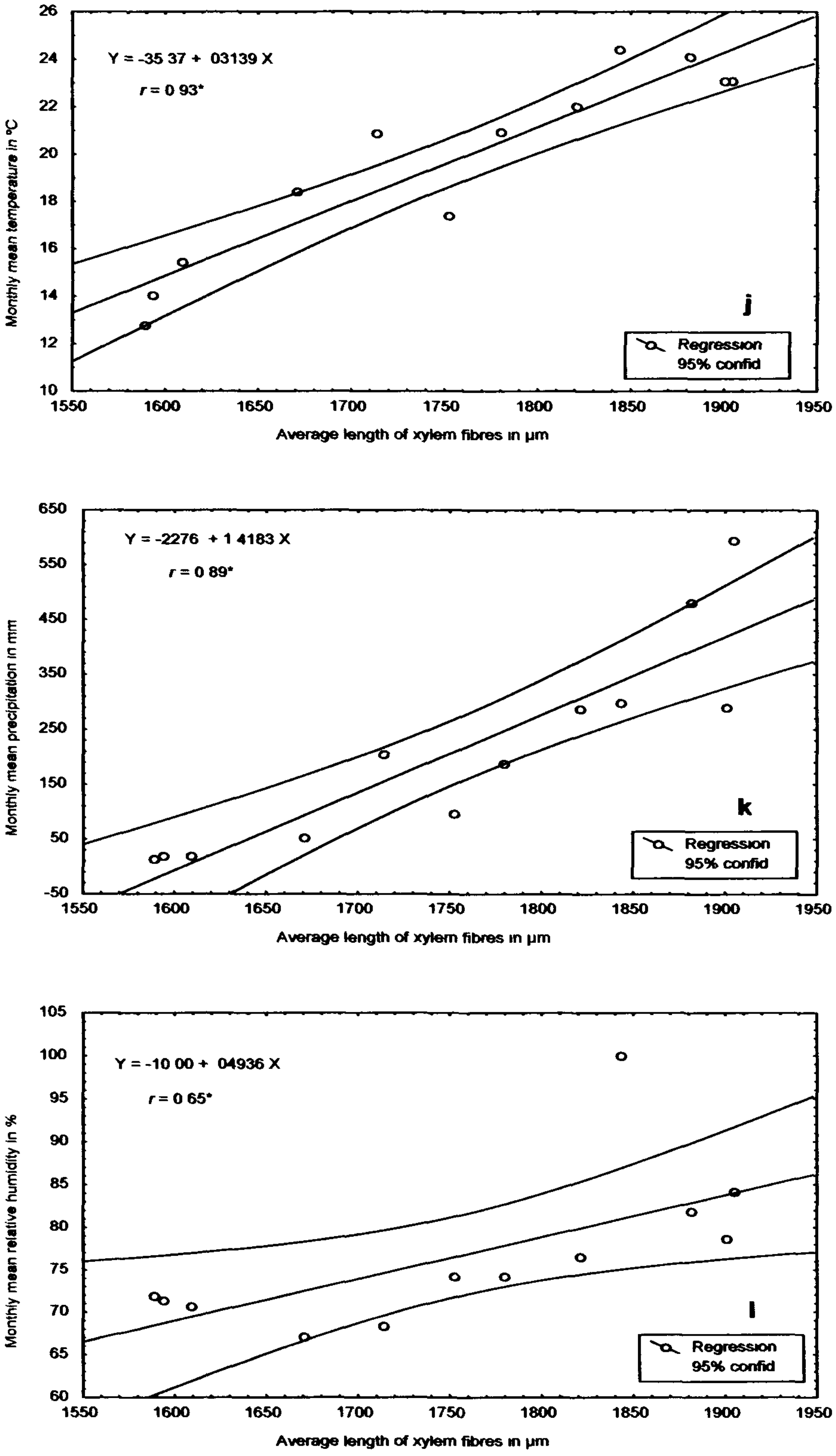
Figs. 5.4 Regression line along with regression equation in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br. All *r values are significant at $p < 0.05$.



Figs. 5.4 Regression line along with regression equation in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br. All $^*r$ values are significant at $p < 0.05$.



Figs. 5.4 Regression line along with regression equation in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br. All *r values are significant at $p < 0.05$.



Figs. 5.4 Regression line along with regression equation in *Exbucklandia populnea* (R. Br. ex Griffith) R.W. Br. All *r values are significant at $p < 0.05$.

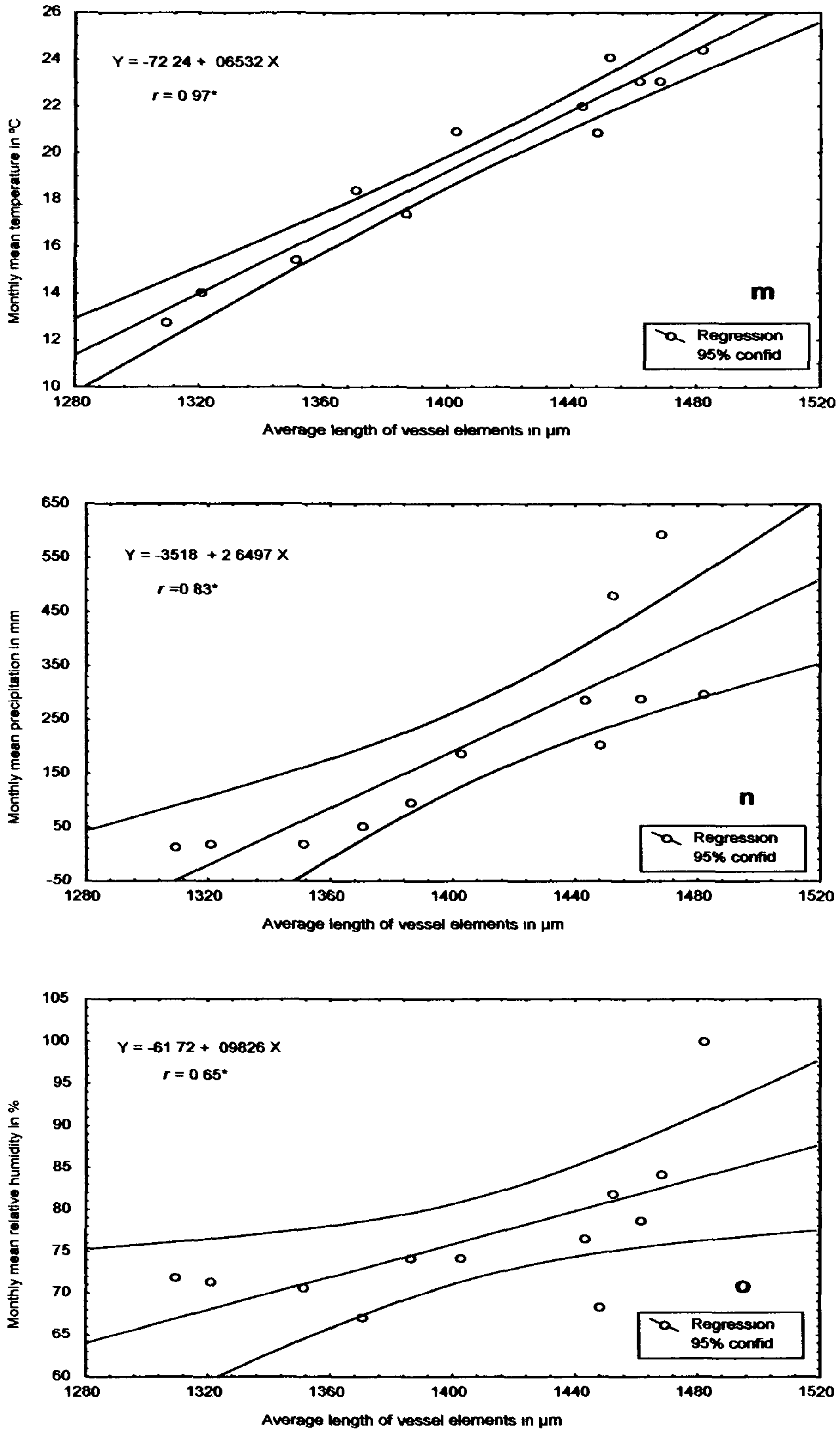


Plate-5.1

a Tangential longitudinal sections (TLS) of dormant cambium showing fusiform initials (Fi) and ray initials (Ri). The beaded appearance is distinct in the radial cell walls. Note the vertical division (Vd) in the one of the ray initial.

b (TLS) of active cambium showing fusiform initials (Fi) and ray initials (Ri). The beaded appearance is not distinct. The nuclei are slender in shape.

c (TLS) showing the elongated nucleus of fusiform initial with two nucleoli (Nuc).

d Transverse section (TS) of dormant cambium. Note the cystolith (Cy), starch grain (S) and phenolic contents (Pc) towards phloem side and in ray initial.

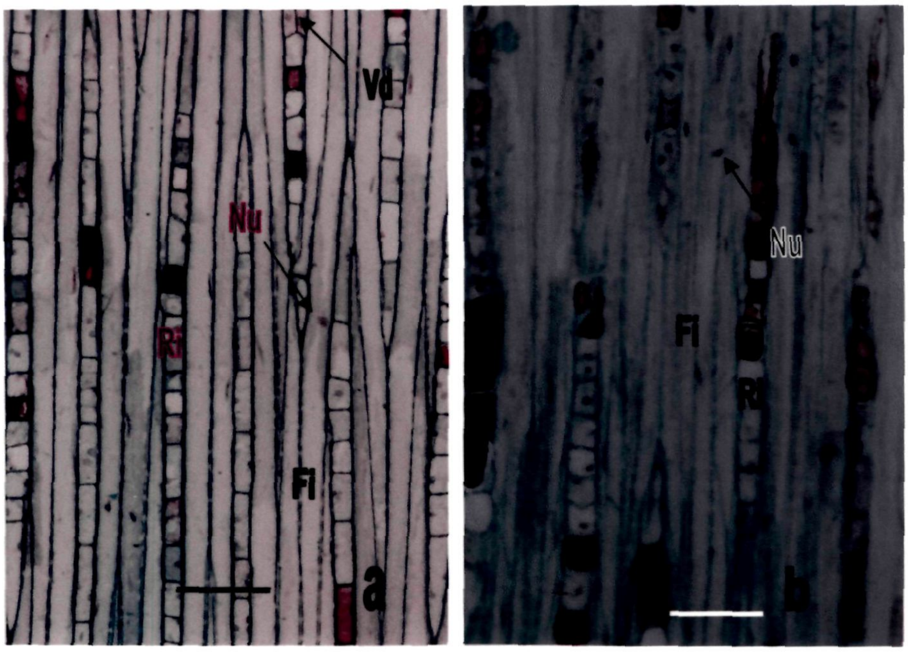
(Bar: **a, b** = 290μm; **c**= 390μm; **d** = 60μm)

Plate- 5.1

Exbucklandia populnea (R. Br. ex Griffith) R.W. Br.

Tangential longitudinal sections of the vascular cambium

Dormant Active



Nucleus with two nucleoli

Transverse section of dormant cambium

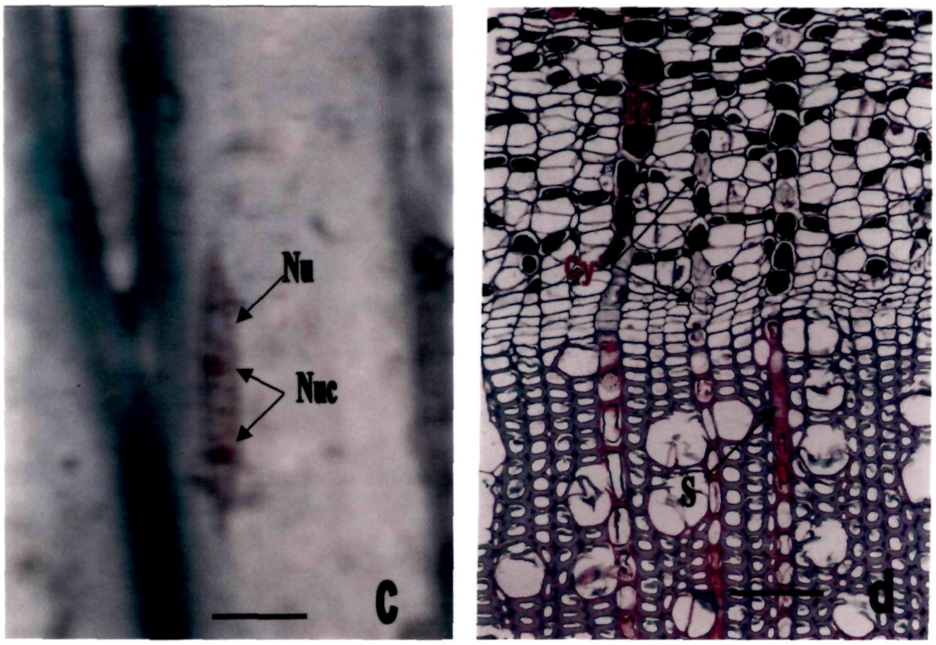


Plate-5.2

a TS of cambium during reactivation in March showing initial periclinal division (Pd) towards the xylem side.

b TS of cambium during the xylem differentiation. Note the increase in number of layers in the cambial zone and deposition of the starch grain (S) in xylem ray and phenolic contents (Pc) on the phloem side.

c TS of the active cambium. The cambial (white arrow) consist of 8-9 layers with differentiating xylem (Dx) and differentiating vessel element (Dv). The cell walls in both the tangential and radial cell wall become much thinner than the dormant one.

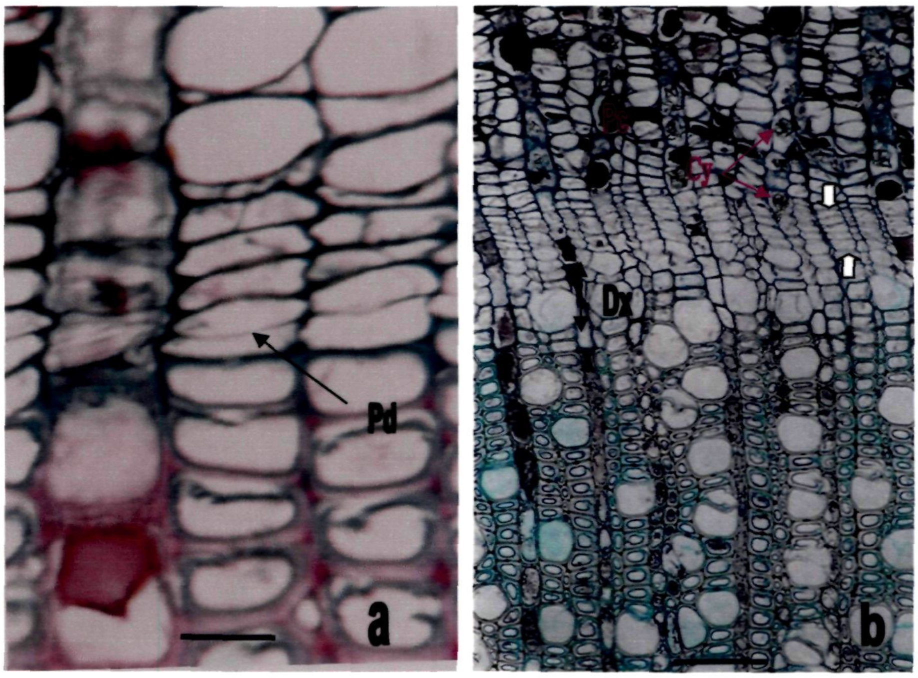
d An enlarged view of active cambial zone in TS.

(Bar: **a** = 40µm **b** = 20µm; **c**= 50µm; **d** = 18µm)

Plate- 5. 2

Exbucklandia populnea (R. Br. ex Griffith) R.W. Br.

Transverse sections during reactivation



Transverse sections of Active cambium

Enlaregd view

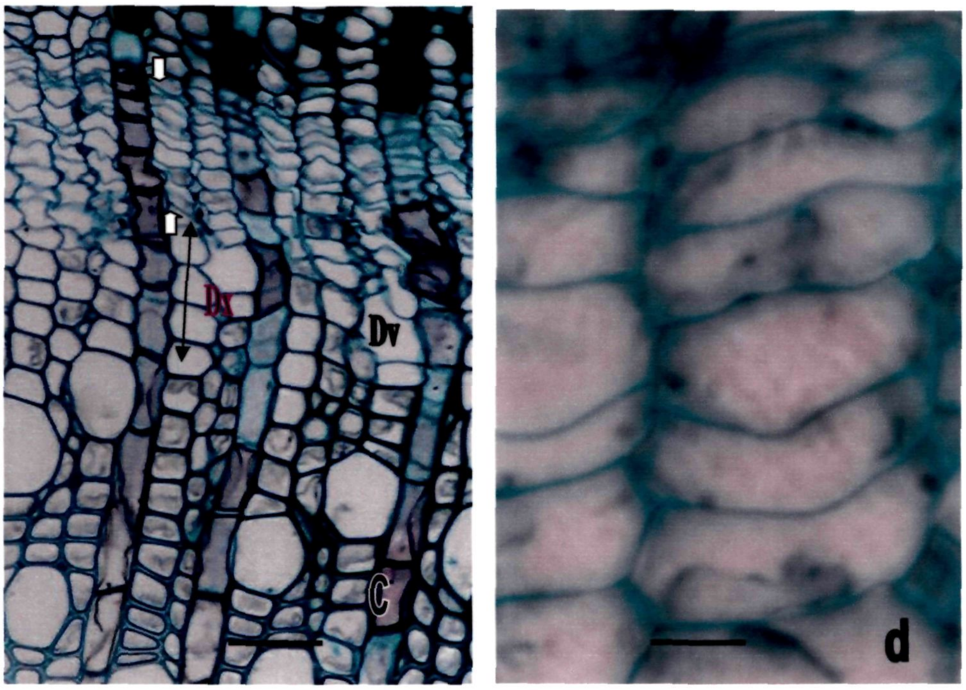


Plate-5.3

a TS of the dormant cambal zone with 3-4 layer (white arrows).

Cystolith (Cs) and phenolic contents (Pc) on the phloem side.

Starch grain (S) in xylem rays.

b Macerated vessel element produced during active period showing the scalariform perforation plate (Sp) with 50 to 60 bars.

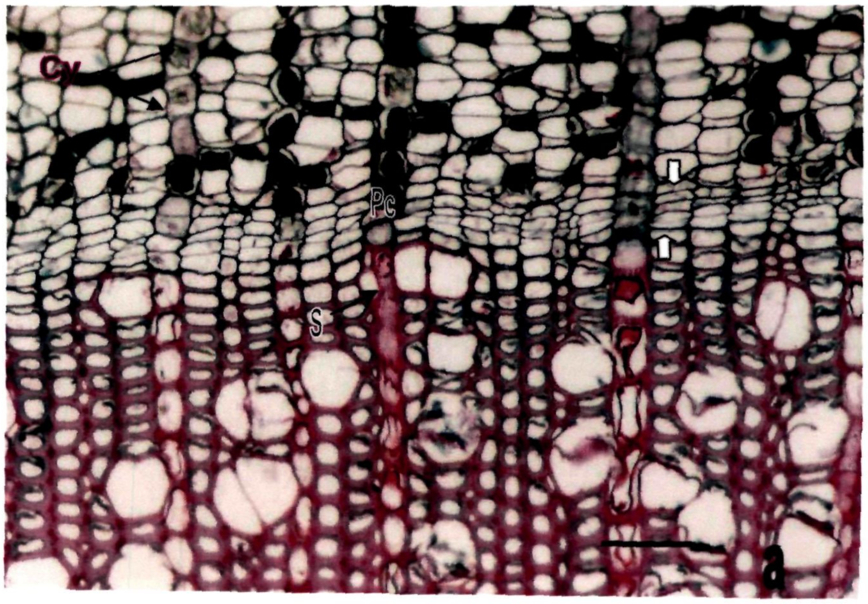
c Macerated late wood vessel element. Scalariform perforation plate with 30 to 40 bars.

(Bar: **a** = 100µm; **b** = 80µm; **c**= 70µm)

Plate- 5. 3

Exbucklandia populnea (R. Br. ex Griffith) R.W. Br.

Transverse section of dormant vascular cambium



Macerated vessel elements

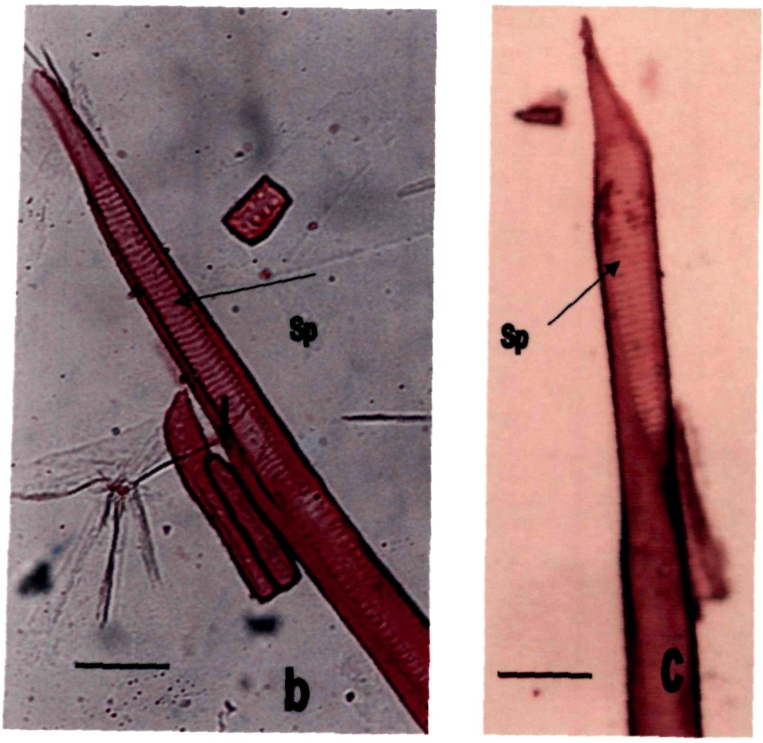


Plate-5.4

a TS of the wood showing indistinct growth rings (white arrow). Xylem fibres (Xf), early wood (Ew), late wood (Lw) and vessel element (Ve) are arranged mostly solitary or in groups of two.

b TLS of wood showing nucleated xylem fibres (Nu).

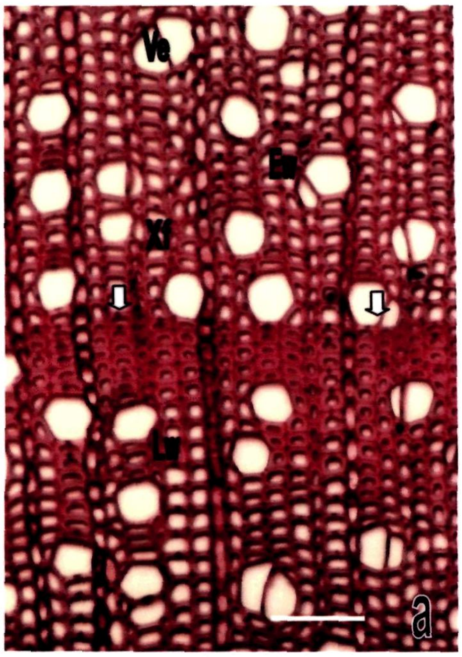
c, d TS of wood showing the inter fibres pits (Fp) in the tangential wall. Note the pit membrane.

(Bar: **a** = 120µm; **b** = 140µm; **c, d** = 40µm)

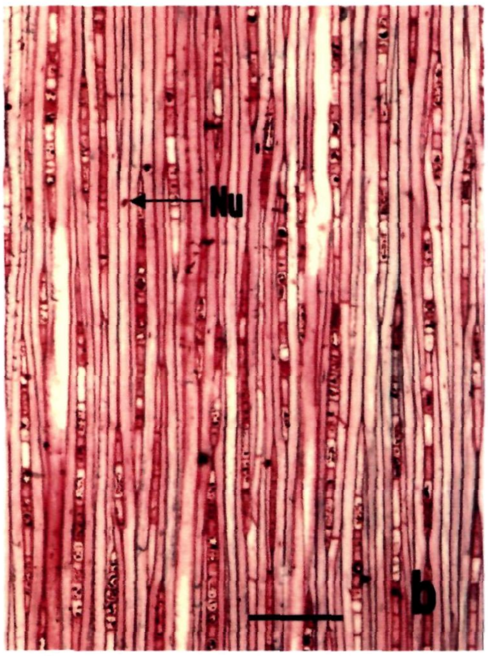
Plate- 5. 4

Exbucklandia populnea (R. Br. ex Griffith) R.W. Br.

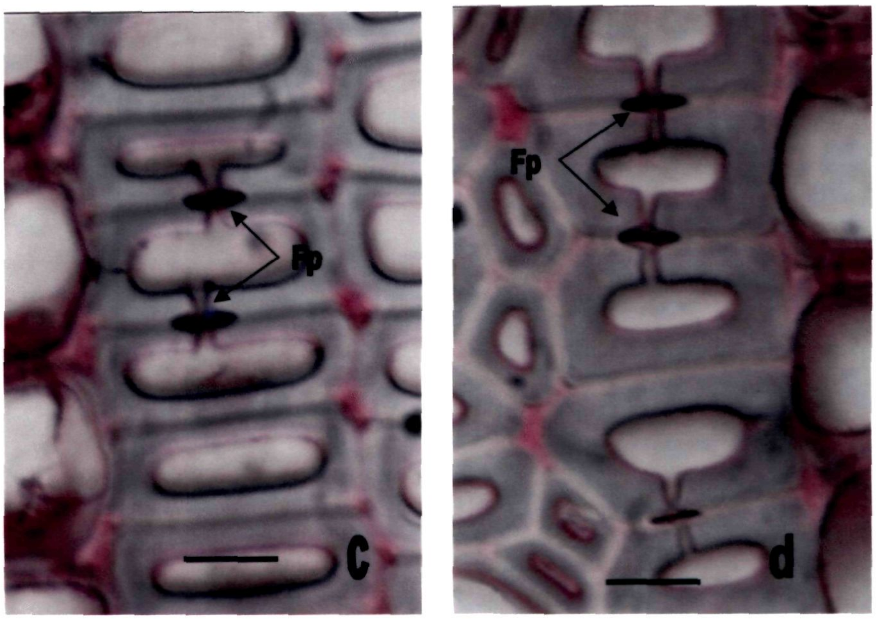
Transverse section of wood



TLS of wood with nucleated xylem fibres



Transverse sections of wood showing inter fibres pits



CHAPTER - 6

***Magnolia grandiflora* Linn.**

Introduction

The genus *Magnolia* was named by Linnaeus in honour of Pierre Magnol. The genus *Magnolia* consists of about 35 species of deciduous or evergreen trees or shrubs. Southern magnolia *(Magnolia grandiflora),* also variously called as evergreen magnolia, sweet magnolia, mountain magnolia, cucumberwood, bull-bay, big-laurel, or large-flower magnolia. The white flowers are large fragrant and evergreen leaves that make it one of the most splendid of forest trees and a very popular ornamental tree which has been planted around the world. Southern magnolia is a fast-growing, medium-sized, evergreen tree that grows 18 to 27m tall. According to geologist, *Magnolia* trees have been on earth for 80 to 100 million years.

Srivastava and Suzuki (2001) have described the fossil wood of *Magnoliaceoxylon palaeogenica* (Magnoliaceae) from the lower Oligocene Tsuyazaki formation in Tsuyazaki. The wood anatomy of many Chinese species of Magnoliaceae was studied in detail by Liang et al. (1993). Carlquist (1988) described the different general feature of wood anatomy of Magnoliaceae in general. He also reported that, in *M. grandiflora,* the vessel elements were arranged in radial multiples and the lateral wall pitting was of scalariform type.

However, the seasonal behaviour of the vascular cambium and differentiation of xylem elements as well as its duration has not been studied in detail in *M. grandiflora*. Therefore, this chapter elucidates the seasonal cambial activity and annual rhythm of xylem production and its duration in relation to phenology and climatic factors in *M. grandiflora*. It will add further information on the behaviour of cambial activity, structure and xylem development of sub-tropical wet forest.

Phenology

Production of new leaves and buds was started in the middle of March and the process of formation of new leaves continued up to the end of November. There was no specific period of leaf fall but a gradual senescence and abscission of older leaves were common during March to June. Leaf fall started at the top of branches and later spread over to the basal ones. The tree never appears leafless during the entire year as new leaves started expanding well before the majority of older leaves have abscised.

The large, white, perfect, showy and fragrant flowers emerge from the tips of twigs on mature trees from the beginning of May and continued up to July. Flowers were terminal, solitary and borne on stout hoary-tomentose pedicels. The fleshy cone like fruit was formed in the months of July and August. When the fruit matures and opens, bright red seeds 6 to 13 mm long emerge from it and seed dispersal took place in the months of September and October. Phenological events like leaf flushes, flowering, fruiting, dispersal of seed and leaf fall were given (Fig. 6.1).

Structure of the vascular cambium and its activity

The vascular cambium was non-storied type in *M. grandiflora*. The fusiform initials were distributed without any tiered arrangement i.e. the tapering ends of fusiform initials lie at different height. The vascular cambium was comprised of vertically elongated fusiform initials and horizontally oriented ray initials (Plate- 6.1a, b). The fusiform initials had two to three sub-globular to elongated nuclei (Plate- 6.1c). The nuclei of each fusiform initial contained one or two nucleoli (Plate- 6.1c, d). The average length of fusiform initials ranged from 945 to 1164 µm and their average width ranged from 18 to 32 µm (Table 6.1). The ray initials were comprised of uniseriate, biseriate and multiseriate rays (Plate- 6.1b). The cell walls of both fusiform and ray initials were thicker with more prominent beaded when the vascular cambium was in dormant condition (Plate- 6.1b). The radial cell walls of the fusiform initials were much thicker than the tangential walls (Plate- 6.2d). On the other hand, during the active period, the cell walls of both fusiform and ray initials were thinner and the beaded appearance was less prominent (Plate- 6.1a).

Cambial activity was initiated in the first week of April which was marked by the radial swelling of fusiform initial and active vacoulation. This was followed by few periclinal divisions in the fusiform initials in the middle of April. Consequently, the number of cambial layers increased to four to five layers and the average width of cambial zone also increased to 64.08 µm in April (Table 6.1). The fusiform initials underwent vertical elongation by intrusive growth and as a result of this process the cell walls became thin and primary pit fields were placed wide apart from each other. Consequently, the beaded appearance became less apparent. The cambial activity then gradually increased and reached its peak in the month of July. The cambial zone was consisted of seven to eight layers in each radial files and average width of cambial zone was 96.12 µm in the month of July (Table 6.1 and Plate- 6.2 a, b). The vascular cambium showed its activity for eight months i.e. from the first week of April to November end (Figs. 6.1 and 6.2a). When the vascular cambium was active, the cambial zone was wider with thin tangential walls and thick radial walls. In other words, the radial walls were thicker than tangential walls. The cambial zone was also surrounded by the differentiating xylem and phloem elements (Plate- 6.2a, b). The average length of fusiform initials varied from 983 to 1164 µm during the active period of the vascular cambium (Table 6.1).

The cambial activity gradually slowed down from October and dormancy was imposed for four months from December to March end (Fig. 6.1). In dormant period, the cambial zone was narrow, consisting of three to four layers and surrounded by mature vascular elements with thick radial and tangential wall as seen in transverse section (Plate- 6.2c, d). The average width of cambial zone was more or less consistent in dormant period of the vascular cambium and varied from 41 to 47 µm (Table 6.1 and Fig. 6.2a). Details pertaining to the number of cambial layers and average width of cambial zone during different months were given (Table 6.1 and Fig. 6.2a).

Differentiation of secondary xylem

Xylem production was noticed only once for nearly seven and half months from the middle of April to November end (Fig. 6.2a and Table 6.1). Xylem production was started with the periclinal division in the vascular cambium, followed by differentiation which resulted in the formation of new xylem fibres, vessel elements, xylem rays and axial parenchyma. Maximum xylem production was noticed from the months of May to September and the average width of differentiating xylem zone ranged from 97 to 133 µm (Table 6.1). During the period of xylem differentiation from the middle of April to November end, the average length of xylem fibres and vessel elements ranged from 1143 to 1385 µm and 843 to 971 µm respectively (Table 6.1). The xylem fibres produced during April were thin walled with larger lumen and angular in transverse section, the cell wall thickness ranged from one and half to two µm (Plate- 6.2a, b) in the differentiating xylem fibres. But the thickness of cell wall of the late wood fibres produced during October and November ranged from seven to nine µm, and the xylem fibres were radially compressed and the lumen was very narrow (Plate- 6.2c). The vessel elements produced during the active period of the vascular cambium had wider lumen and scalariform perforation plates with 15 to 21 bars (Plate- 6.3a); whereas those produced during October and November had narrow lumen and its scalariform perforation plate had 5 to 11 bars only (Plate- 6.3b). Data pertaining to the average width of differentiating xylem zone, initiation of xylem production, cessation as well as its duration of xylem production were given (Table 6.1 and Fig. 6.2a).

A comparison was made between the average length of xylem fibres and vessel elements with that of average length of fusiform initials in different months. The average length of xylem fibres ranged from 1122 to 1385 µm and average width ranged from 23 to 37 µm. The average length and width of vessel varied from 811 to 971 µm and from 44 to 71 µm respectively. The xylem fibres increased in length of 18% over the fusiform initials but the vessel elements showed decreasing trend of about 18% over the fusiform initials. The xylem fibres width showed a marginal increase of about 2% over the width of vessel elements. On the other hand, the width of vessel elements showed an increase of about 12% over the width of fusiform initials. The variations in average length of xylem fibres and vessel elements was more or less corresponded with the changes in average length of fusiform initials in all the months during the study period 2002-2003. The data pertaining to the vascular cambium and its derivatives were given (Table 6.1 and Figs. 6.1; 6.2a, b).

Structure of secondary xylem

The secondary xylem of *M. grandiflora* was consisted of xylem fibres, xylem rays, vessel elements, axial and terminal parenchyma cells (Plate-6.3c). The wood was diffuse porous, evenly textured, moderately heavy, fairly hard and straight grained. The sapwood of *Magnolia* was creamy white, while the heartwood was light to dark brown, often with greenish to purple-black streaks or patches. Heartwood was hardly distinguishable from the sapwood.

The axial parenchyma was apotracheal solitary and diffuse in aggregate (Plates- 6.2c and 6.3c). The vessel elements were arranged solitary or in radial multiples. The end wall of vessel elements showed scalariform perforation plate. The lateral walls pittings of vessel elements was of scalariform type and helical thickening were present in the vessel elements (Plate- 6.3c). The xylem rays were uniseriate, biseriate and multiseriate. Growth rings were distinct and it was demarcated by the presence of three to four wide terminal parenchyma (Plate- 6.3c).

The starch grains were the major food reserve and were distributed in phloem parenchyma, phloem rays, xylem rays, xylem parenchyma when the vascular cambium was in dormant condition (Plate- 6.2c). At the time of initiation of cambial activity the amount of starch grain were reduced. However, starch grains were not present during the peak period of the vascular cambium.

Cambial activity in relation to phenology

Phenological observation clearly indicated that the cambial activity in *M. grandiflora* was correlated with phenological events. Sprouting of new leaves and buds began in the middle of March. In the first week of April i.e. two weeks after the development of new leaves and buds, the fusiform initials showed the sign of radial swelling and active vacoulation. This was followed by periclinal divisions in the middle of April and as a result, the number of cambial layers increased to four to five layers in April (Table 6.1). The process of formation of new leaves continued up to end of November, and during this period i.e. from

April to November, the number of cambial layers ranged from four to eight layers. The flowering was noticed from May to July and fruiting occurred in July and August and during this flowering and fruiting season i.e. from May to August, the number of cambial layers ranged from six to eight. Seed dispersal took place in the months of September and October. There was no specific period of leaf fall but a major leaf fall was observed continuously from March to June. The vascular cambium remains dormant for four months from December to March end (Fig. 6.1). In *M. grandiflora*, there was no correlation between leaf fall and cambial dormancy because young leaves emerged in concurrence with the shedding of old leaves.

Relationship between climatic factors, cambial activity and xylem production

The various climatic factors during the study period of the year 2002-2003 were given in Figure 6.3 (see data in Materials and Methods). When the cambium reactivation and differentiation of xylem elements began from April to November end, a strong positive correlation was observed with monthly mean ambient temperature. The correlation coefficient (*r*) of the average width of cambial zone (*r*= 0.90) (Fig. 6.4a); average width of differentiating xylem zone (*r*= 0.94) (Plate- 6.3g); average length of fusiform initials (*r*= 0.92) (Plate- 6.3d); average length of xylem fibres (*r*= 0.81) (Plate- 6.3j) and average length of vessel elements (*r*= 0.87) (Plate- 6.3m) with monthly mean temperature were high (Table 6.2). The correlation coefficient (*r*) and (*t*) values of different anatomical variables such as average width of cambial zone, average width of differentiating xylem zone, average length of fusiform initials, xylem fibres and vessel elements versus different climatic factors such as monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity were given in Table 6.2. The (*r*) values of cambial parameter such as the average width of cambial zone, the average length of fusiform initials, xylem fibres and vessel elements with that of different climatic factors such as monthly mean, mean minimum, mean maximum temperature, precipitation and relative humidity were significant. However, the average width of differentiating xylem zone showed significant (*r*) values with the monthly mean, mean maximum, mean minimum temperature and monthly rainfall only and did not showed significant (*r*) value with monthly mean relative humidity (Table 6.2). All these relationships were high explaining more than 40% of the variability between two variables.

Therefore, multiple (partial) regression analysis was carried out to find out how much each one of the climatic parameters contributes to the cambial activity of the vascular cambium. It was evident from the multiple (partial) regression analysis, that monthly mean temperature was statistically significant with the average width of cambial zone ($t \geq +2.65$), average width of differentiating xylem zone ($t \geq +3.36$). The correlation coefficients of the average width of cambial zone ($r = 0.90$) (Fig. 6.4a) and the average width of differentiating xylem zone ($r = 0.94$) (Plate- 6.3g) with the monthly mean temperature were also high. The average length of vessel elements ($t \geq +3.24$) showed statistically significant relationship with monthly mean rainfall.

However, the average length of fusiform initials, and xylem fibres did not show any statistical significant (*t*) values with any one of the climatic parameters. The monthly mean minimum ambient temperature had statistically significant inverse relationship with the average width of cambial zone ($t \geq -2.47$). It was evident from correlation and multiple (partial) regression analysis; monthly mean temperature was an important factor for cambial activity and differentiation of xylem elements. There was not much significant effect of rainfall and relative humidity on cambial activity and xylem production.

Fig. 6.1 Phenology, duration of cambial activity and dormancy in *Magnolia grandiflora* Linn.

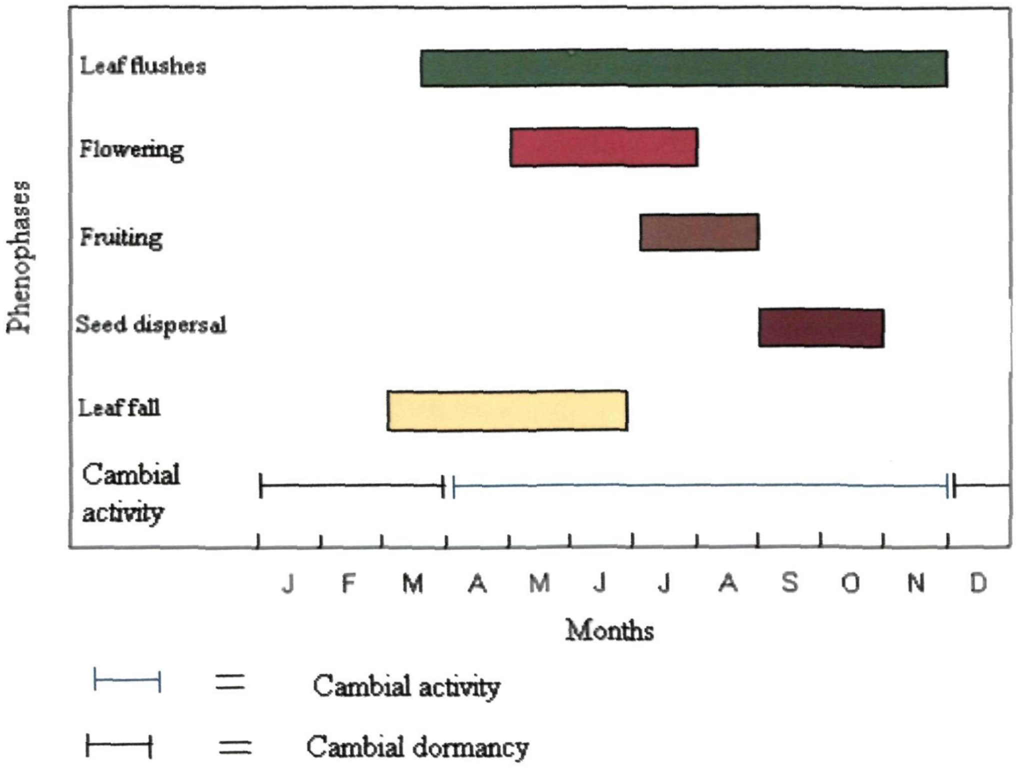


Table 6.1 Average quantitative data of the vascular cambium and its derivatives in *Magnolia grandiflora* Linn.

Months	Numbers of cambial layers	Average width of cambial zone in µm.	Average width of differentiating xylem zone in µm.	Average length of fusiform initials in µm.	Average length of xylem fibres in µm.	Average length of vessel elements in µm.
February	3-4	45.15 ±8.21	—	951.24 ±17.10	1122.72 ±45.35	811.36 ±59.54
March	3-4	46.10 ±9.07	—	945.13 ±12.04	1125.47 ±51.23	825.04 ±60.20
April	4-5	64.08 ±5.65	61.11 ±10.24	1086.17 ±20.09	1238.63 ± 37.68	870.45 ±35.03
May	5-6	73.03 ±10.76	99.04 ±8.90	1107.53 ± 20.16	1318.19 ±53.72	925.25 ±34.25
June	6-7	86.13 ±10.80	121.05 ±17.42	1142.37 ±13.25	1338.63 ±57.40	970.45 ±52.22
July	7-8	96.12 ±6.97	132.03 ±12.04	1163.28 ±15.52	1384.09 ±45.10	959.09 ±61.51
August	6-7	89.05 ±8.11	101.02 ±14.01	1124.87 ±19.58	1327.26 ±51.78	920.45 ±36.77
September	5-6	72.04 ±7.62	97.06 ±11.68	1091.28 ±20.22	1188.63 ±64.57	897.72 ±37.83
October	4-5	54.05 ±7.25	43.04 ±5.91	1048.34 ±28.21	1165.90 ±50.33	870.45 ±47.19
November	4-5	53.14 ±8.57	40.11 ±9.45	983.27 ±15.52	1143.18 ±54.87	843.18 ±25.22
December	3-4	43.06 ±9.09	—	953.08 ±12.68	1129.54 ±40.02	818.19 ±17.07
January	3-4	41.04 ±6.35	—	952.04 ±24.81	1131.81 ±54.87	825.70 ±53.61

— = No xylem production
± = Standard deviation

Table 6.2 The correlation coefficients (*r*) and (*t*) values of the vascular cambium and its derivatives versus different climatic factors in *Magnolia grandiflora* Linn.

	Monthly mean temperature in°C		Monthly mean maximum temperature in°C		Monthly mean minimum temperature in°C		Monthly mean precipitation in mm		Monthly mean relative humidity in %	
	r	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Average width of cambial zone in µm	0.90*	+2.65*	0.83*	-2.13	0.91*	-2.47*	0.92*	+2.01	0.77*	+1.71
Average length of fusiform initials in µm	0.92*	+1.23	0.86*	-1.16	0.93*	-0.86	0.92*	+1.07	0.66*	-0.20
Average width of differentiating xylem zone in µm	0.94*	+3.36*	0.70*	-1.99	0.86*	-2.14	0.88*	+1.26	0.54	-1.30
Average length of xylem fibres in µm	0.81*	+1.09	0.74*	-0.64	0.82*	+1.31	0.89*	+1.81	0.68*	+0.91
Average length of vessel elements in µm	0.87*	+0.95	0.79*	-1.11	0.90*	-0.55	0.97*	+3.24*	0.67*	+0.24

All **r* and **t* values are significant at $p < 0.05$.

Figs. 6.2. Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Magnolia grandiflora* Linn.

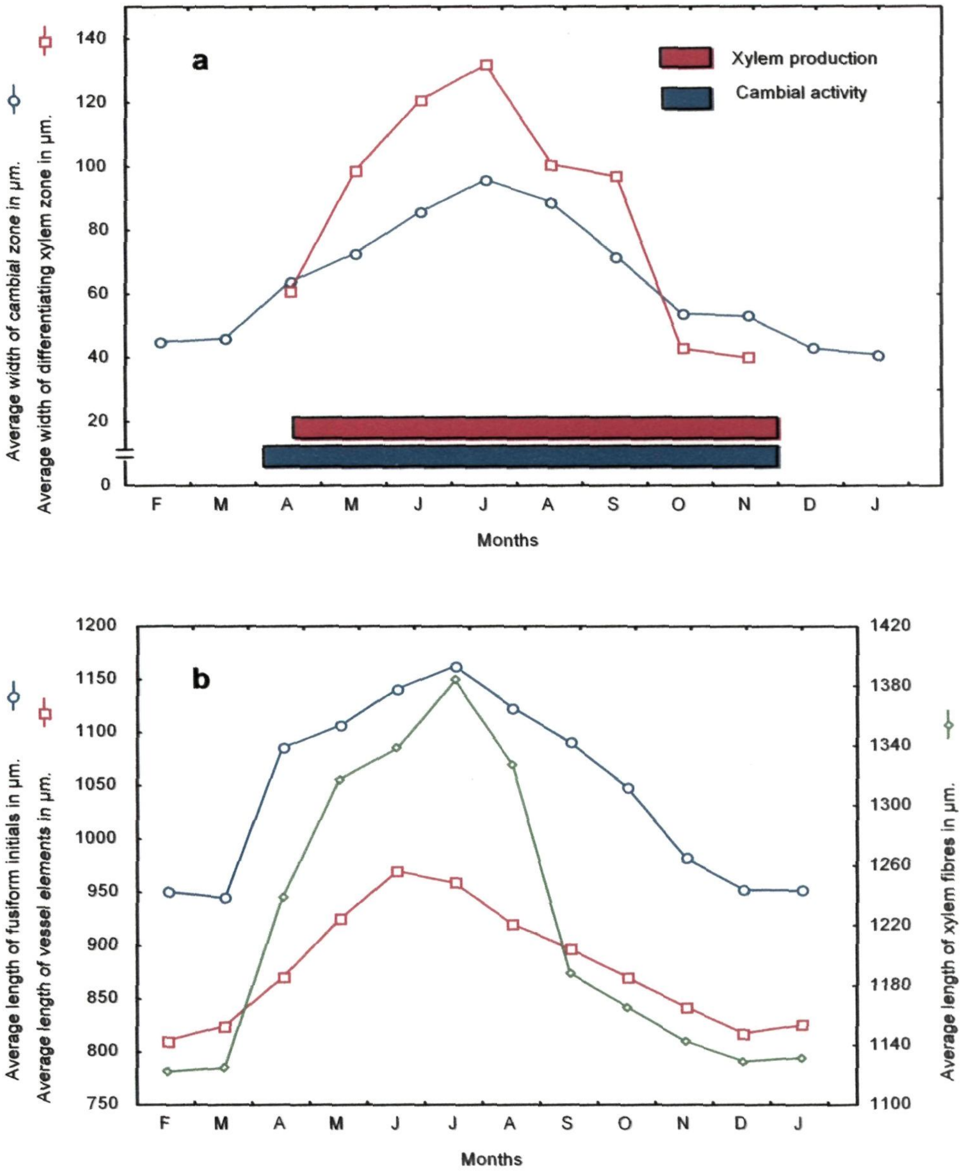
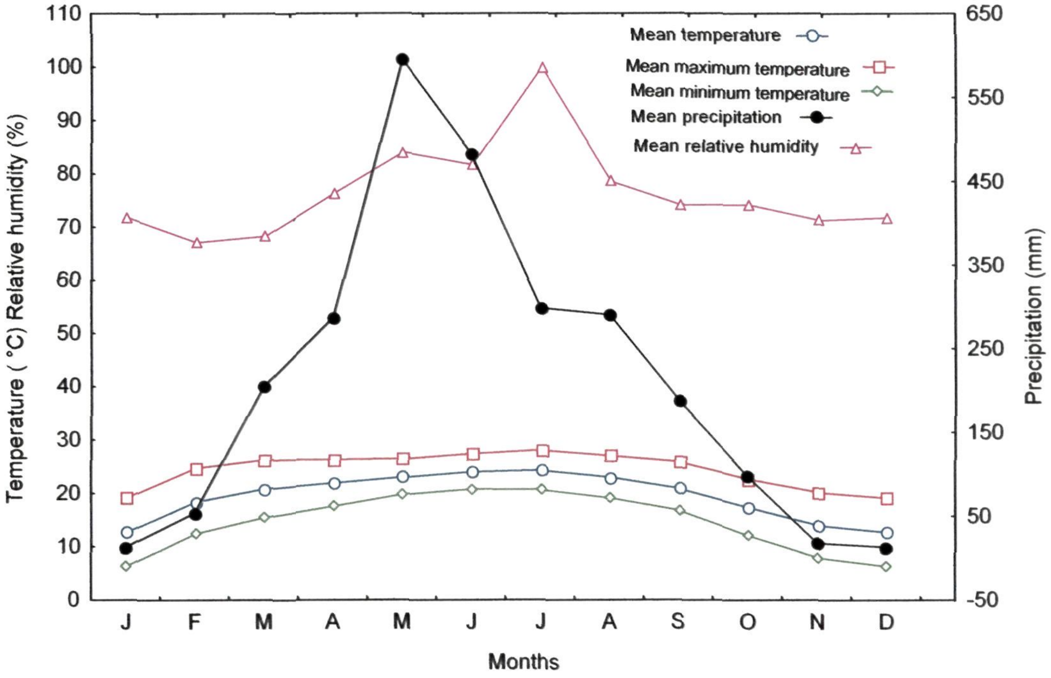
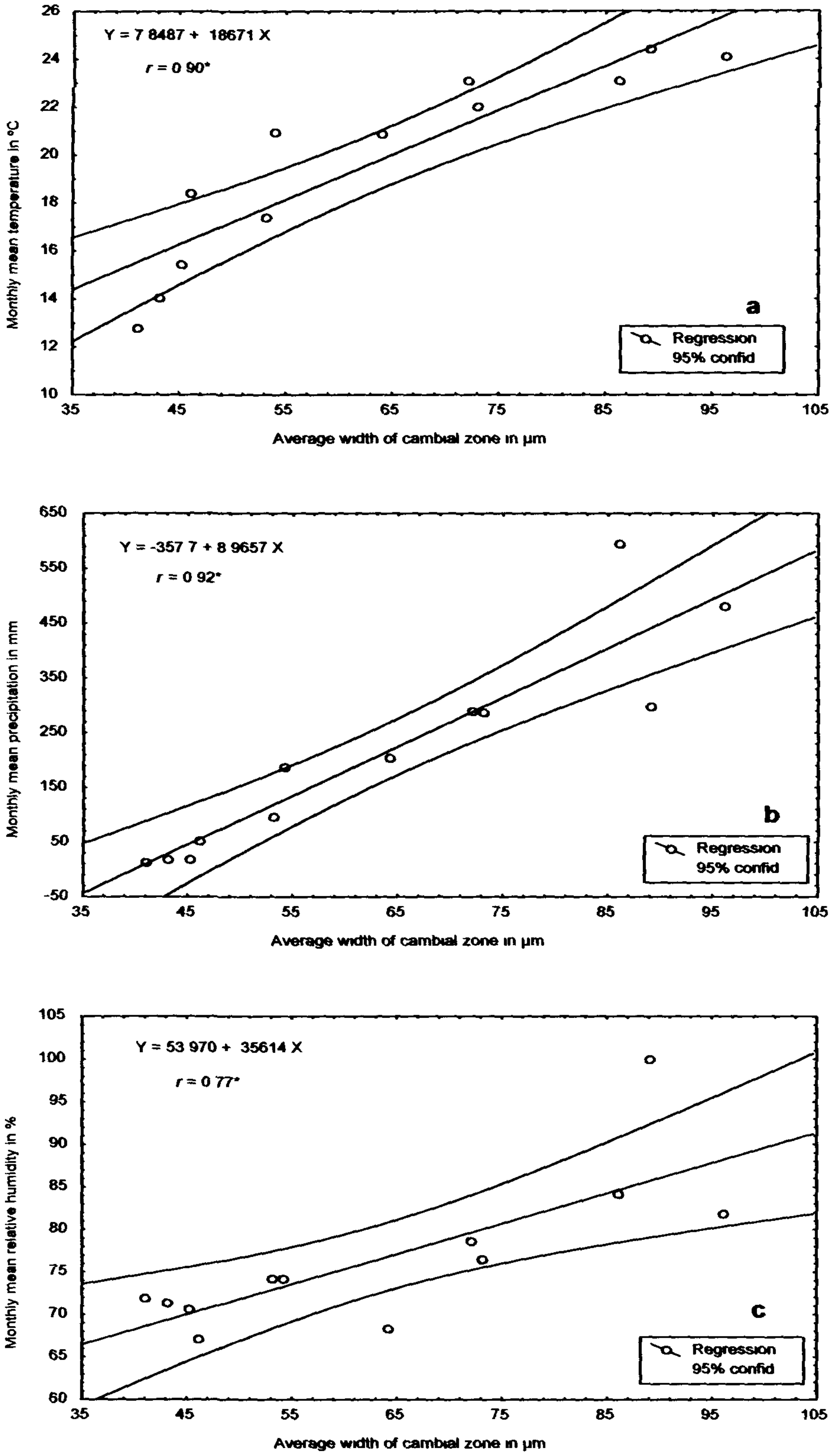


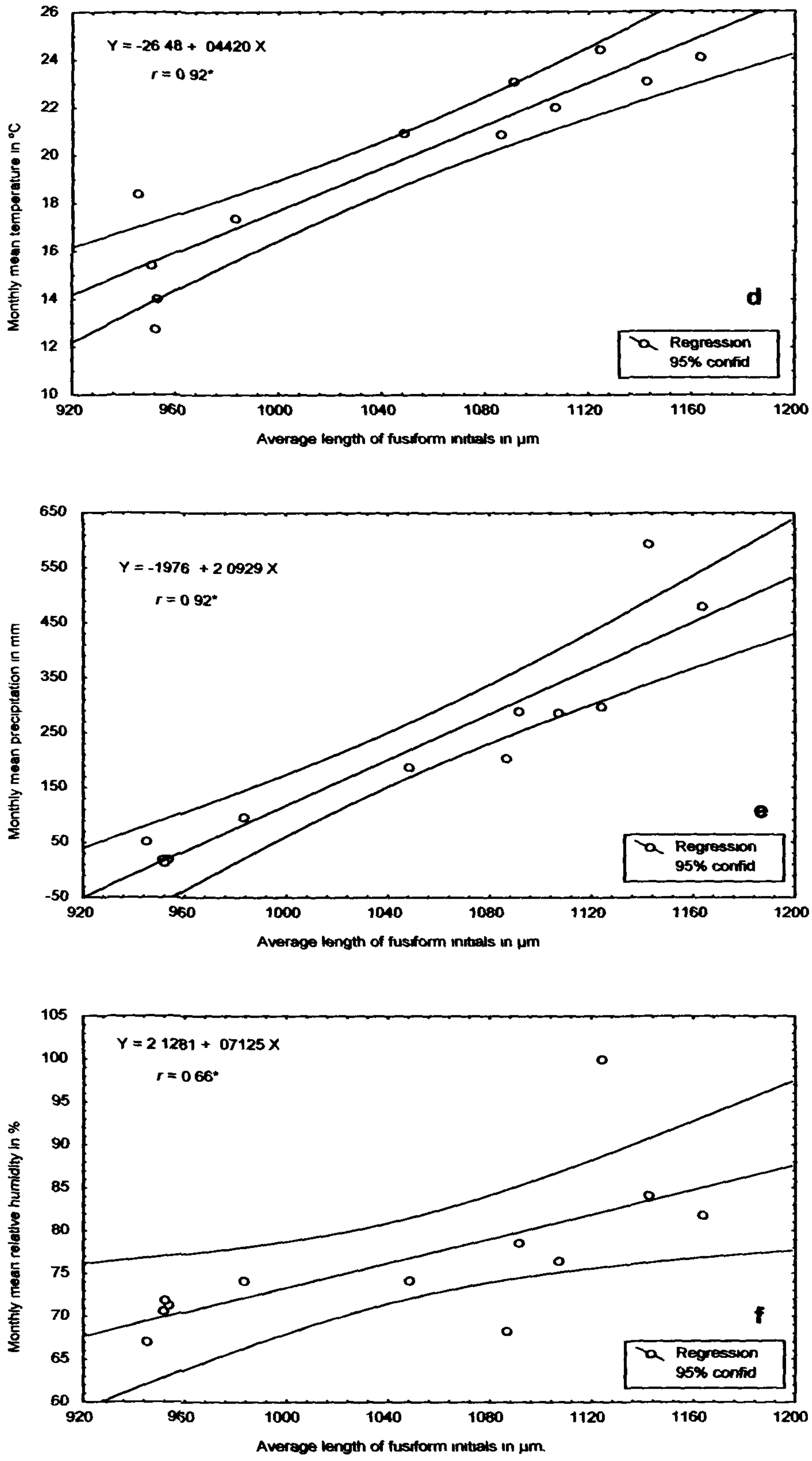
Fig. 6.3 Graphical representation of average climatic data of the years 2002-2003.



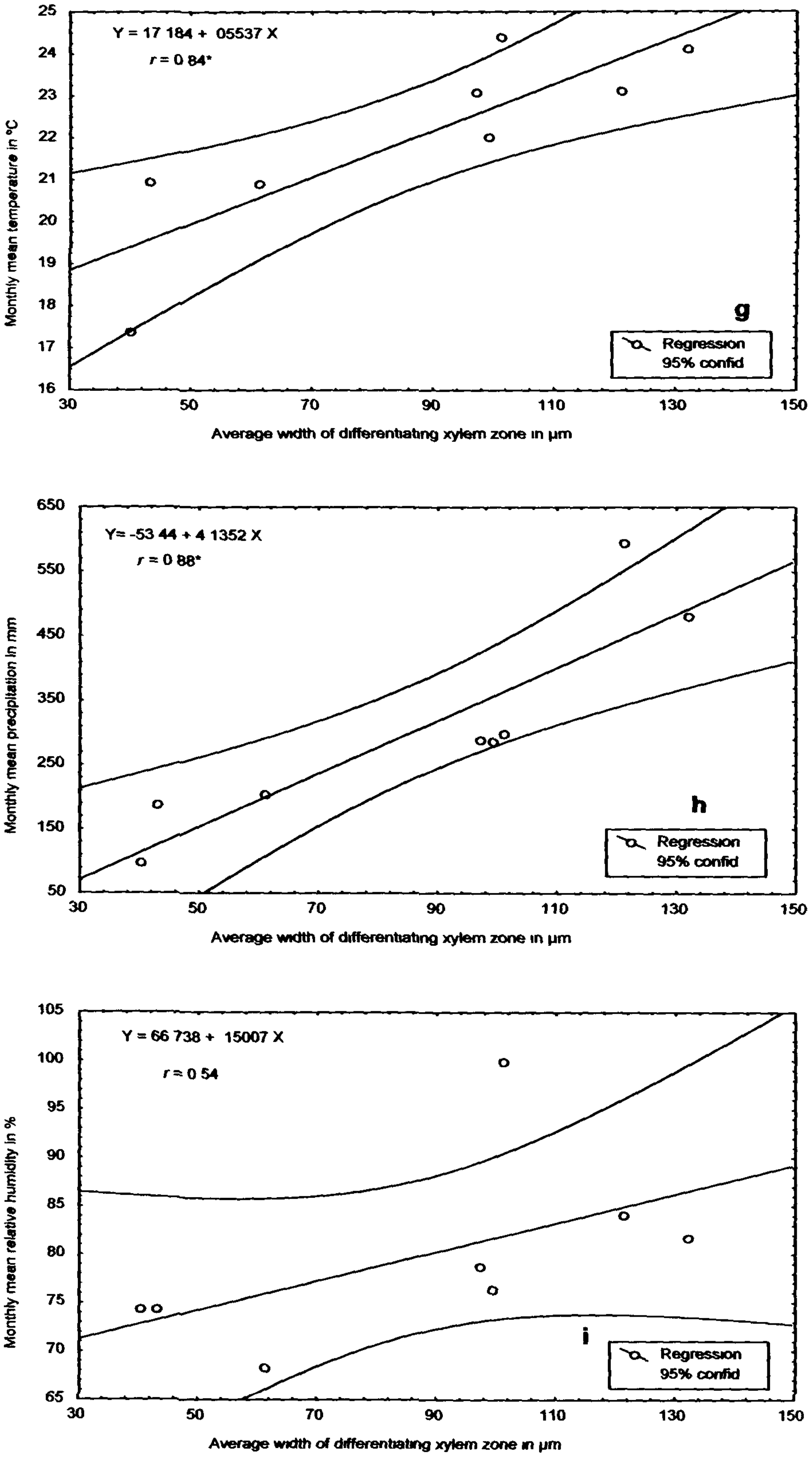
Figs. 6.4 Regression line along with regression equation in *Magnolia grandiflora* Linn. All *r values are significant at $p < 0.05$.



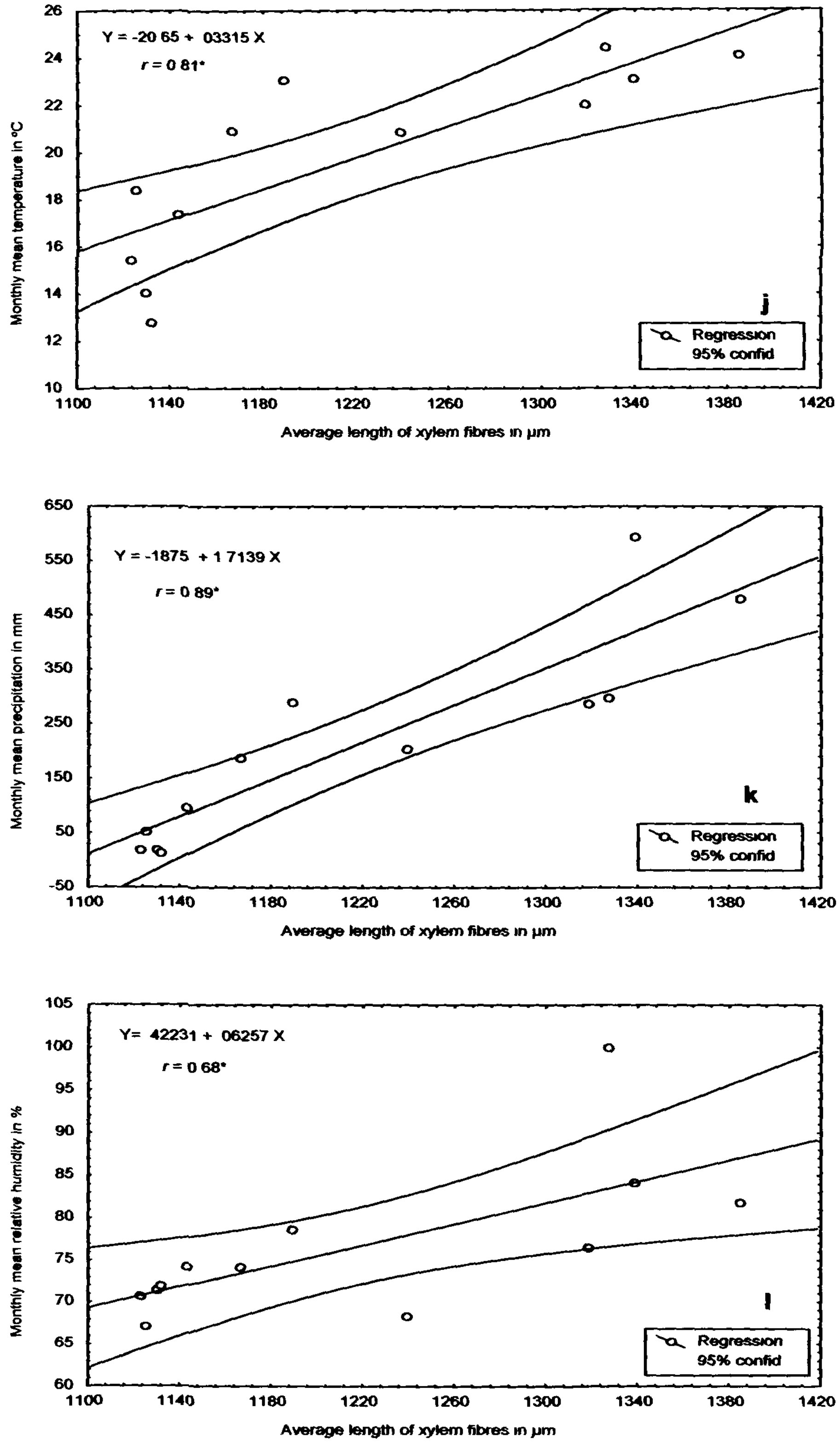
Figs. 6.4 Regression line along with regression equation in *Magnolia grandiflora* Linn. All *r values are significant at $p < 0.05$.



Figs. 6.4 Regression line along with regression equation in *Magnolia grandiflora* Linn. All *r values are significant at $p < 0.05$.



Figs. 6.4 Regression line along with regression equation in *Magnolia grandiflora* Linn. All *r values are significant at $p < 0.05$.



Figs. 6.4 **Regression line along with regression equation in *Magnolia grandiflora* Linn. All *r values are significant at $p < 0.05$.**

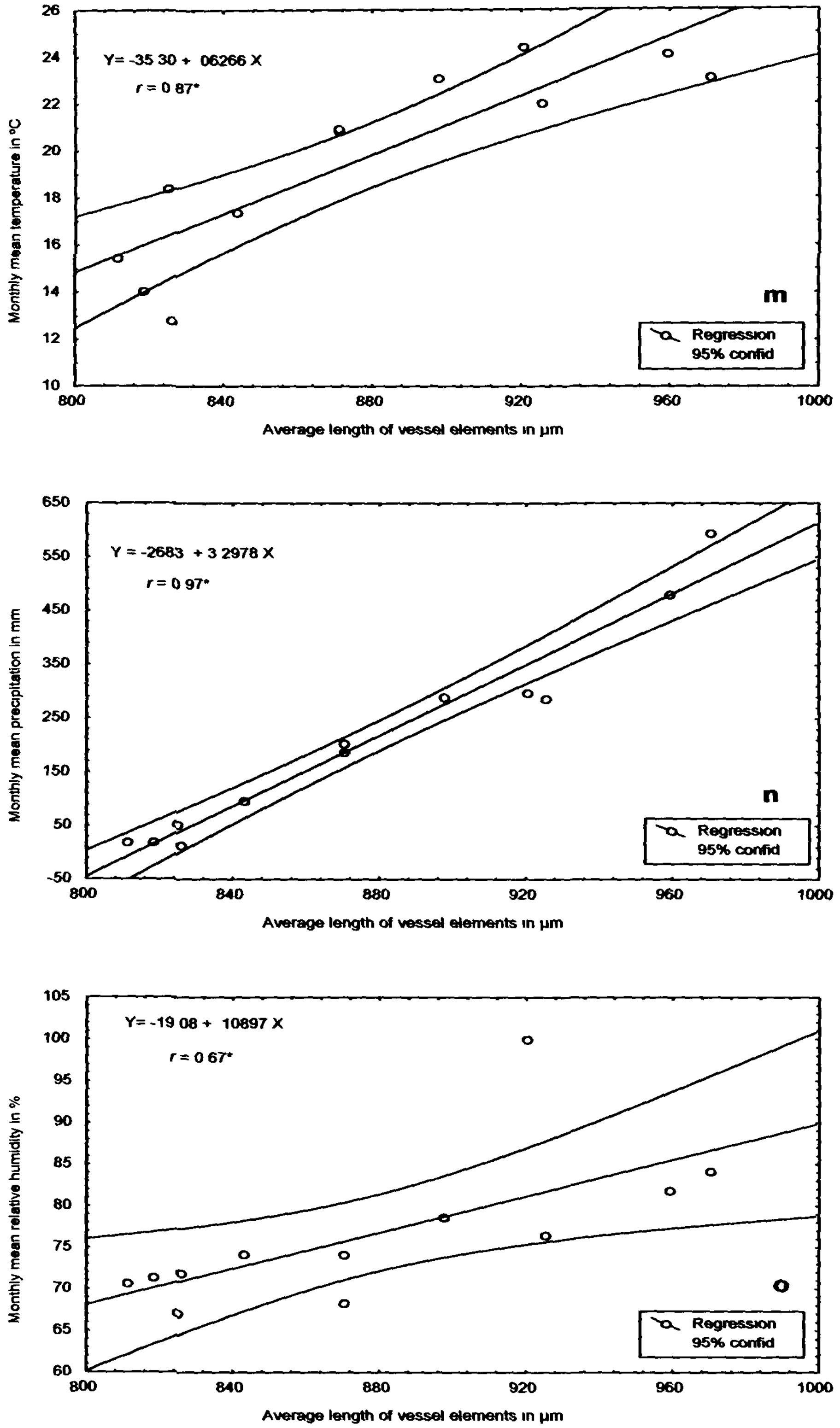


Plate- 6.1

a Tangential longitudinal sections (TLS) of active cambium showing fusiform (Fi) and ray initials (Ri). The beaded appearance is not prominent in the fusiform initials; but in differentiating phloem the beads are distinct. Note the multiseriate (Mr), biseriate (Br) and uniseriate (Ur).

b (TLS) of dormant cambium with fusiform (Fi) and ray initials (Ri). The bead nature of cell wall is prominent. Note the multiseriate (Mr), biseriate (Br) and uniseriate (Ur) ray initials.

c (TLS) showing two nuclei (Nu) in the fusiform initials, which are elongated or sub-globular in shape.

d Nucleus with two nucleoli (Nuc) in the differentiating phloem cells.

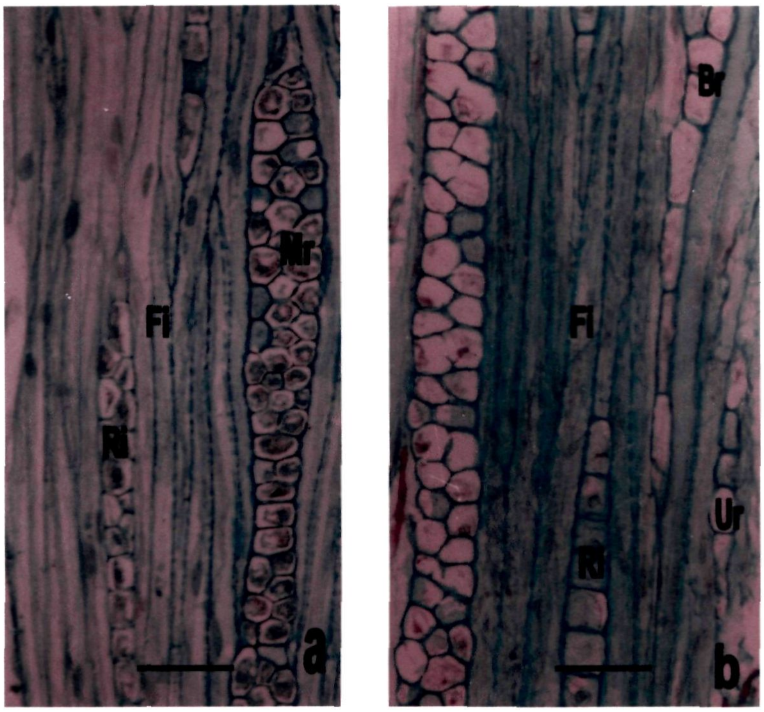
(Bar: **a, b** = 155µm; **c**= 60µm; **d** = 80µm)

Plate- 6.1

Magnolia grandiflora Linn.

Tangential longitudinal sections of the vascular cambium

Active Dormant



Tangential longitudinal sections of cambium

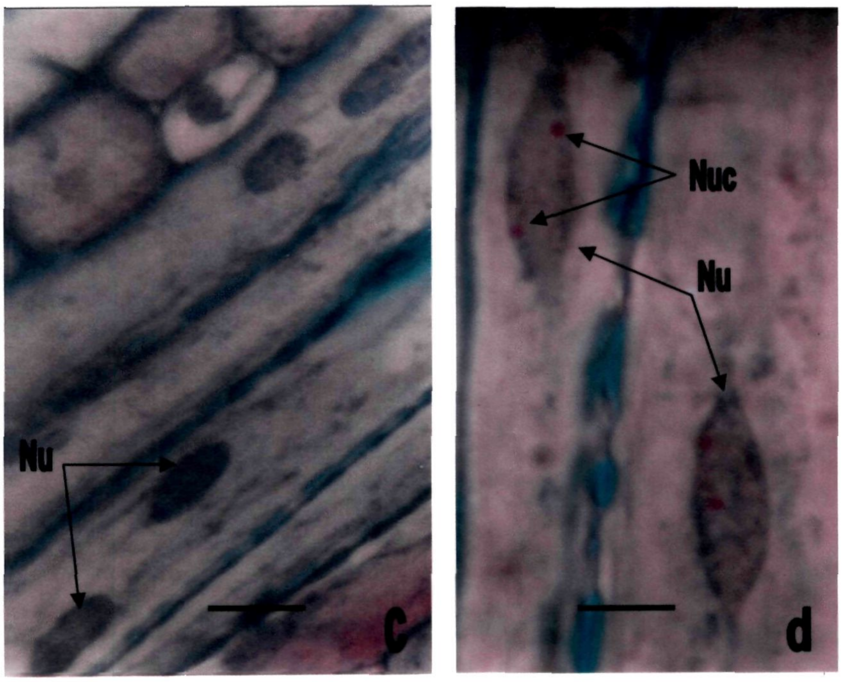


Plate- 6.2

a Transverse sections (TS) of active cambium. The cambial zone (white arrow) consists of 7-8 layers with differentiating xylem (Dx) and differentiating vessel elements (Dv).

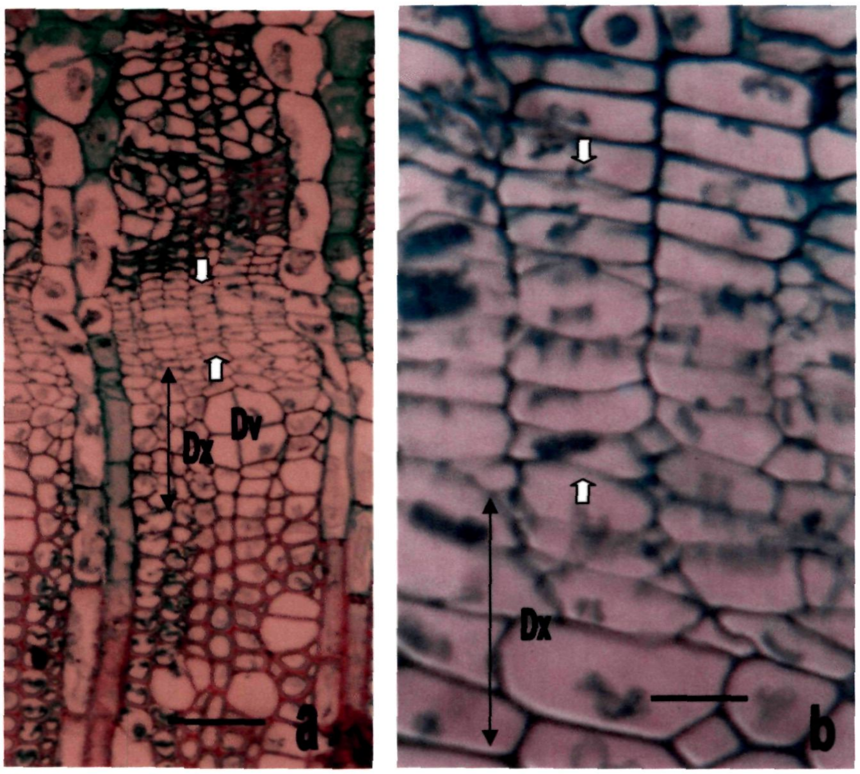
b Enlarged view of (**a**). The cambial zone (white arrow) consists of 7-8 layers with differentiating xylem (Dx). Note the formation of new rays in the cambial zone and in some of the fusiform initials nuclei are prominent as well as the thickness of the cell walls are thinner than the dormant one

c, d TS of dormant cambium showing 3-4 layers (white arrow). Starch grains(S) are deposited as dark structures in the xylem rays and fibres. Note the apotracheal banded xylem parenchyma.

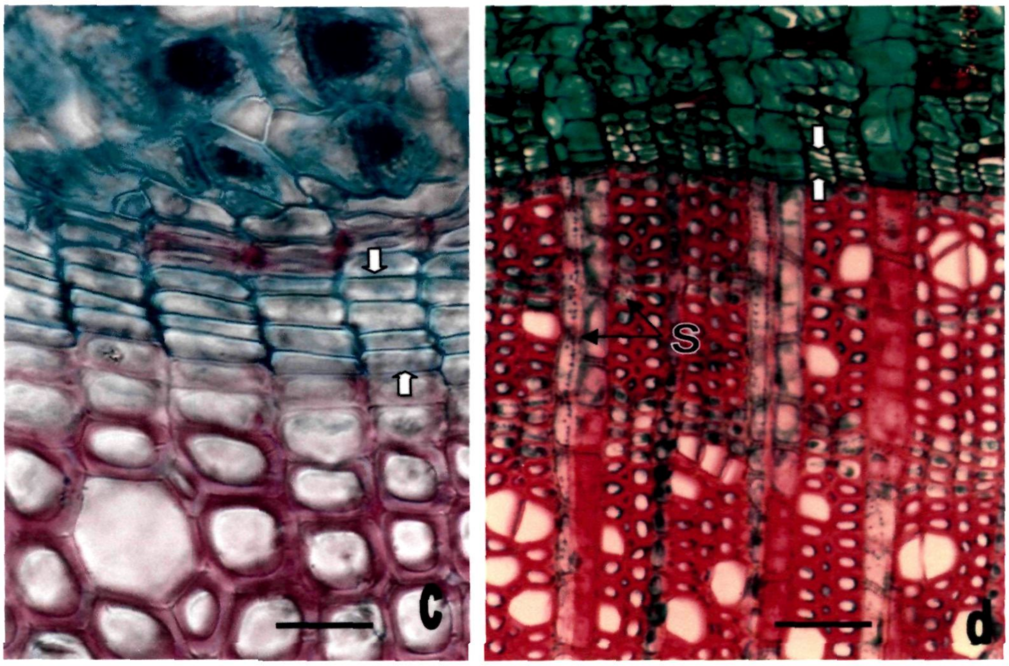
(Bar: **a** = 120µm; **b** = 40µm; **c**= 60µm; **d** = 180µm)

Plate- 6.2

Magnolia grandiflora Linn.



Transverse sections of active cambium



Transverse sections of active cambium

Plate-6.3

a Macerated vessel elements produced during the active period. Scalariform perforation plate (Sp) with 15 bars. Note the helical cell wall thickening (Ht) also.

b Macerated late wood vessel elements. Scalariform perforation plate (Sp) with 11 bars. Note the helical thickening (Ht).

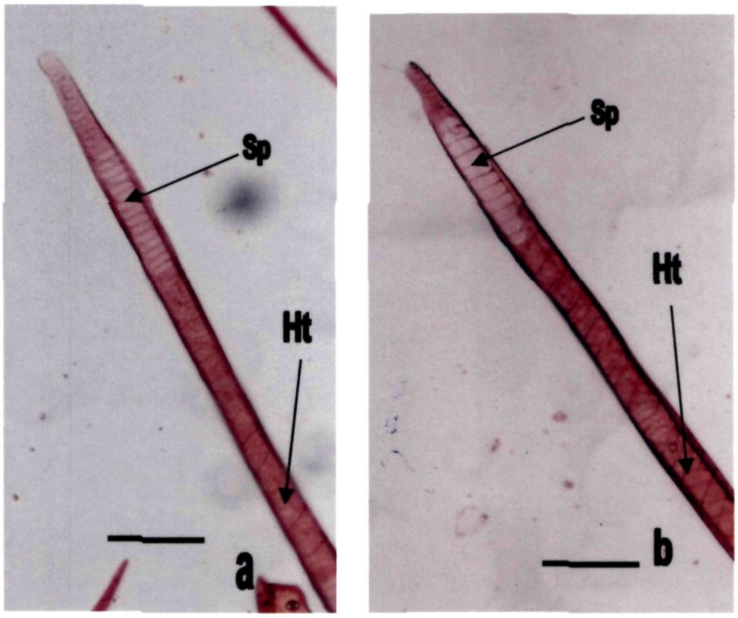
c Transverse section (TS) of wood. Growth ring distinct (white arrow) with terminal parenchyma: xylem fibres (Xf), early wood (Ew), late wood Lw). Vessel element (Ve) mostly solitary and occasionally 2 or 3.

(Bar: **a** = 176µm; **b** = 135µm; **c**= 250µm)

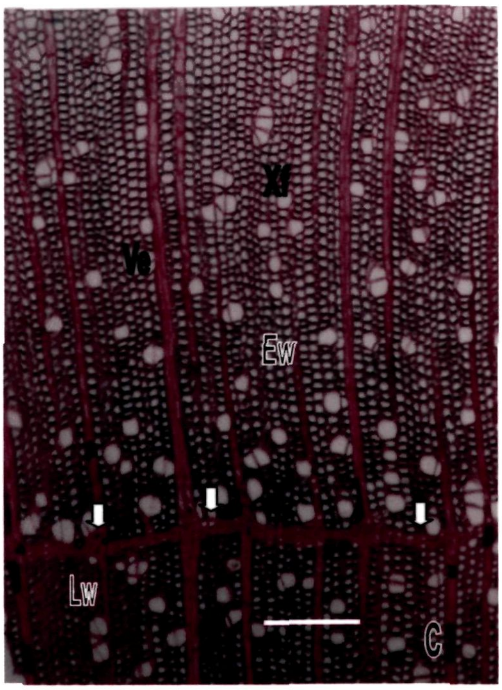
Plate- 6.3

Magnolia grandiflora Linn.

Macerated vessel elements



Transverse section of active cambium



CHAPTER - 7

***Michelia champaca* Linn. and *Michelia oblonga* Wall. ex Hk.f.**

Introduction

The genus *Michelia* was named after the Florentine botanist Peter Antonio Michel. The genus includes about 50 species, native to tropical and subtropical south and southeast Asia (Indomalaya), including southern China. The genus *Michelia* is very closely related to sub-genus *Yualania* of genus *Magnolia,* based on morphological characteristic (Figlar 2000) and molecular data (Azuma et al. 1999; Kim et al. 2001).

Monteiro (2002) studied the phenological aspects of *M. champaca* growing in southeastern Brazil. The wood structure of eight Indian species of *Michelia* (*M. oblonga*, *M. champaca*, *M. manii*, *M. nilagirica*, *M. bailonii*, *M. kisopa*, *M. lanuginosa* and *M. excelsa*) was studied in detail (Chauhan et al. 1992). The wood structure and its physical and mechanical properties of *M. champaca* were described by (Pearson and Brown 1981). Several species of *Michelia* are locally important sources of timber yielding trees in Meghalaya.

The present study was aimed to understand the seasonal activity of the vascular cambium and annual rhythm of xylem production in relation to phenology and climatic factors in *M. champaca* and *M. oblonga*, growing in sub-tropical moist forest of Meghalaya.

Phenology

In *M. champaca* and *M. oblonga,* the annual growth in terms of elongation of the branches apices commonly consist of new apical buds being formed from the middle of February. The process of formation of new leaves and buds continued up to October and November in *M. oblonga* and *M. champaca* respectively. Leaf senescence was initiated from November in *M. oblonga* and December in *M. champaca*. In both species leaf fall continued up to the end of January. The old leaves abscised before the arrival of new leaves and the tree remain barren for nearly 15 to 20 days; therefore, *M. champaca* and *M. oblonga* belong to brevi-deciduous type (Borchert 1999).

Flowering began from the month of June up to August in both species. The blossoms of *Michelia* generally form clusters among the leaves. The fruit formation was seen from August and mature fruit were borne in the months of August and September in *M. champaca* and *M. oblonga*. Fruiting was followed by seed dispersal which took place in the months of September and October in both species (Figs. 7.1A and 7.1B).

Structure of vascular cambium and its activity

The vascular cambium of *M. champaca* and *M. oblonga* was non-storied and comprised of two cells types- long, tangentially flattened fusiform initials and horizontally arranged isodiametric ray initials. In *M. champaca* and *M. oblonga,* the fusiform initials contained three to four nuclei which were spherical or elongated in shape (Plate- 7.1a, b, c, d). Each nucleus of the fusiform initials had one or two nucleoli in both species (Plate- 7.1a, d). The ray initials were comprised of uniseriate, biseriate and multiseriate types (Plate- 7.1a, b, c). The average length fusiform initials ranged from 907 to 1103 µm and 928 to 1185 µm in *M. champaca* and *M. oblonga* respectively

(Table 7.1A and 7.1B). The average width of fusiform initials varied from 19 to 35 µm in *M. champaca* and 20 to 31 µm in *M. oblonga*. In both species, the cell walls of fusiform initials were thin and the beaded appearance was not prominent during the active period (Plate- 7.1a, c). In contrast, the cell walls of fusiform initials were thick and the characteristics beaded appearances were more prominent in the dormant cambium (Plate- 7.1b, d).

Reactivation of cambium was seen after completion of dormancy in the first week of March in both the species. Cambial reactivation was marked by the radial swelling (Plate- 7.2a) and the change in vacuolar system of the fusiform initials. In both the species, the fusiform initials underwent few periclinal divisions from the middle of March onwards (Plate- 7.2a, a_1, a_2). As a result of these periclinal divisions, the number of cambial layers increased to four to five layers in the month of March in both the species. Consequently, the average width of cambial zone also increased to 60.45 µm in *M. champaca* and to 56.16 µm in *M. oblonga* in March (Table 7.1A and 7.1B). The cambial activity was then gradually increased and reached its peak in the month of June with eight to nine layers and the average width of cambial zone was 102.04 µm in *M. champaca* (Plate- 7.3a and Table 7.1A). While, in *M. oblonga,* the vascular cambium showed maximum activity in July with eight to nine layers and the average width of cambial zone was 100.21 µm (Plate- 7.3b, c and Table 7.1B). The vascular cambium showed its activity for eight months from the first week of March to the end of October in *M. oblonga* and for nine months i.e. from the first week of March to the end of November in *M.*

champaca (Figs. 7.1A, 7.1B and Figs. 7.2A, 7.2B). The cambial zone was wider and surrounded by differentiating xylem and phloem elements during the active period of the vascular cambium (Plate- 7.3 a, b, c). The length fusiform initials on average ranged from 930 to 1103 µm in *M. champaca* and from 991 to 1185 µm in *M. oblonga* when the vascular cambium was in active period (Table 7.1A and 7.1B).

The cambial activity gradually slowed down from September to October in *M. oblonga* and from October to November in *M. champaca*. During these months, the fusiform initials showed septate condition i.e. approaching towards dormancy. Therefore, xylem fibres derives from such septate fusiform initials also showed septate condition in both the species (Plates- 7.4a_1, a_2, 7.1d). In *M. champaca,* the cambium remained dormant for three months from December to the end of February (Fig. 7.1A). However, the cambium was in dormant condition for four months from November to February end in *M. oblonga* (Fig. 7.1B). When the cambium was in dormant condition, the cambial zone was consisted of three to four layers in each radial files in both the species (Table 7.1A, 7.1B; Plates- 7.4a and 7.5a). The cambial zone was narrow and surrounded by thick walled xylem and phloem elements in dormant cambium (Plates- 7.4a and 7.5a). The average width of cambial zone was more or less consistent during dormant period and it ranged from 40 to 48 µm in *M. champaca* and from 42 to 49 µm in *M. oblonga* (Table 7.1A and 7.1B). The cambial zone variability (number of cambial layers and average width of cambial zone) was shown (Figs. 7.2A, 7.2B; Table 7.1A and 7.1B).

Differentiation of secondary xylem

Xylem production began with periclinal divisions in the vascular cambium and was observed from the middle of March in both the species (Figs. 7.2A and 7.2B). It resulted in the formation of new xylem fibres, vessel elements, xylem rays and parenchyma. Xylem differentiation continued up to October end and November end in *M. oblonga* and *M. champaca* respectively. Xylem production was seen for about eight and half months in *M. champaca* and about seven and half months in *M. oblonga* (Figs. 7.2A and 7.2B). Maximum xylem production was seen from April to September in *M. champaca* and the average width of differentiating xylem zone varied from 107 to 161 µm, whereas in *M. oblonga* maximum xylem production was observed from May to September and the average width of differentiating xylem zone ranged from 99 to 138 µm (Table 7.1A and 7.1B). In both the species, the fibres produced during March were thin walled with larger lumen and angular in transverse section, the cell wall thickness ranged from two to four µm, (Plate- 7.3a, b) whereas, the cell walls thickness of the late wood fibres ranged from 9 to 11 µm, and the fibres were radially compressed and the lumen was very narrow (Plates- 7.4a and 7.5a).

During the period of xylem production, the average length of xylem fibres ranged from 1086 to 1289 µm in *M. champaca* and 1090 to 1407 µm in *M. oblonga* (Table 7.1A and 7.1B). In *M. champaca,* the vessel elements produced during the active period had wider lumen and scalariform perforation plates with six to nine bars, whereas those produced during October and

November had the scalariform perforation plate had three or four bars (Plate- 7.5a, b). In *M. oblonga,* the vessel elements produced during the active period had scalariform perforation plates with six to ten bars with wider lumen, whereas those produced during September and October had narrow lumen and scalariform perforation plate with four to five bars (Plate- 7.6a, b). The average length of vessel elements varied from 570 to 776 µm in *M. champaca* and from 654 to 828 µm in *M. oblonga* during the period of xylem differentiation (Table 7.1A and 7.1B). Details regarding the average width of differentiating xylem zone, initiation and cessation of xylem production as well as its duration were given (Figs. 7.2A, 7.2B; Table 7.1A and 7.1B).

A comparison was made between the average length of fusiform initials, xylem fibres and vessel elements. In *M. champaca,* the average length and width of xylem fibres ranged from 1011 to 1289 µm and 22 to 36 µm respectively (Table 7.1A). Whereas, in *M. oblonga,* the average length of xylem fibres varied from 1018 to 1407 µm and width of xylem fibres from 17 to 35 µm (Table 7.1B). The average length of vessel elements was found to vary from 529 to 776 µm and 615 to 828 µm in *M. champaca* and *M. oblonga* respectively (Table 7.1A and 7.1B). The width of vessel elements on average ranged from 37 to 71 µm in *M. champaca* and 35 to 69 µm in *M. oblonga.*

The length of xylem fibres shows an increase of about 14 % from that of fusiform initials in *M. champaca* and *M. oblonga.* The increased in width of xylem fibres was only 0.74% in *M. champaca* and 0.20% in *M. oblonga* from that of fusiform initials. There was a decrease in length of vessel elements by

54% in *M. champaca* and 46% in *M. oblonga* when compare to the length of fusiform initials. In both species, the width of vessel elements showed an increase of about 10% from the width of fusiform initials. In *M. champaca* and *M. oblonga,* the variation in average length of xylem fibers and vessel elements undergo same sequence of variation with that of fusiform initials during different months. The seasonal variations in average length of fusiform initials, xylem fibres and vessel elements in different months of the years 2002-2003 are shown (Figs. 7.3A, B).

Structure of secondary xylem

The secondary xylem of both *M. champaca* and *M. oblonga* was diffuse porous and consisted of xylem fibres, vessel elements, xylem rays and axial parenchyma. In *M. champaca* the sapwood is white; the heartwood is light yellowish-brown to olive-brown and somewhat lustrous, without characteristic odour or taste. The growth rings were distinct in both the species, being delimited by light lines of initial parenchyma in *M. champaca* and *M. oblonga* (Plate- 7.6 c, d). The vessels were mostly solitary or in radial multiple of two to three and distributed randomly in both species. The end wall of vessel elements had scalariform perforation plate and the lateral wall pitting was of scalariform type in both the species (Plates- 7.5b, c and 7.6a, b).

The starch grains were the major food reserve and were distributed in phloem parenchyma, phloem rays, xylem rays, xylem parenchyma and occasionally in xylem fibres during dormancy (Plates- 7.4a and 7.5a). At the time of initiation of cambial activity the amount of starch grain were reduced. However, starch grains were not observed during the peak activity of the vascular cambium.

Cambial activity in relation to phenology

In *M. champaca* and *M. oblonga,* sprouting of young new leaves and buds began in the middle of February. Initiation of cambial activity was seen in the first week of March, nearly 15 to 20 days after the formation of new leaves and buds (Figs. 7.1A and 7.1B). In both the species, cambial reactivation was followed by periclinal division in the middle of March and as a result, the number of cambial layers increased to four to five layers in both *M. champaca* and *M. oblonga* (Table 7.1A and 7.1B). A positive correlation exists between cambial reactivation and leaf emergence. Sprouting of new leaves and buds continued up to November in *M. champaca* and during this period the numbers of cambial layers in the cambial zone ranged from four to nine whereas in *M. oblonga,* the process of formation of new leaves continued up to October and the numbers of cambial layers ranged from four to nine. From the months of June to August i.e. during flowering period in both species the number of cambial layers ranged from six to nine in *M. champaca* and seven to nine in *M. oblonga.* The number of cambial layers varied from six to seven in *M. champaca* and from six to eight in *M. oblonga* during the period of fruiting period in the months of August and September. When seed dispersal took place in the months of September and October, the variability in cambial layer was five to seven and four to seven in *M. champaca* and *M. oblonga* respectively. Leaf senescence was initiated from November in *M. oblonga* and from December in *M. champaca* and leaf fall continued up to the end of

January. During the period of leaf fall, the number of cambial layers ranged from three to four in both the species. In *M. champaca* and *M. oblonga,* there was a positive correlation between leaf fall and cambial dormancy (Figs. 7.1A, 7.1B).

Relationship between climatic factors, cambial activity and xylem production

The different climatic factors during the study period of the year 2002-2003 are given (Fig. 7.4; Data in Materials and Methods). During the onset of cambial reactivation and the differentiation of xylem elements from the first week of March to October end in *M. oblonga*, November end in *M. champaca*, a strong positive correlation was observed with monthly mean ambient temperature. The correlation coefficient (*r*) of the average width of cambial zone (*r*= 0.94) (Fig. 7.5Aa); average width of differentiating xylem zone (*r*= 0.93) (Fig. 7.5Ag); average length of fusiform initials (*r*= 0.90) (Fig. 7.5Ad); average length of xylem fibres (*r*= 0.85) (Fig. 7.5Aj) and average length of vessel elements (*r*= 0.92) (Fig. 7.5Am) with monthly mean temperature were high in *M. champaca*. Similarly in *M. oblonga*, the correlation coefficient (*r*) of the average width of cambial zone (*r*= 0.90) (Fig. 7.5Ba); average width of differentiating xylem zone (*r*= 0.84) (Fig. 7.5Bg); average length of fusiform initials (*r*= 0.89) (Fig. 7.5Bd); average length of xylem fibres (*r*= 0.90) (Fig. 7.5Bj) and average length of vessel elements (*r*= 0.88) (Fig. 7.5Bm) with monthly mean temperature were also high.

In *M. champaca,* the average width of cambial zone, average length of vessel elements showed significant (*r*) values with all the climatic factors but the average width of differentiating xylem zone, average length of fusiform initials and average length of xylem fibres did not showed significant (*r*) values with monthly mean relative humidity (Table 7.3A). The average width of cambial zone, average length of fusiform initials, average length of xylem fibres and average length of vessel elements showed significant (*r*) values with all the climatic factors but the average width of differentiating xylem zone did not show significant (*r*) values with relative humidity in *M. oblonga* (Table 7.3B). All these relationships were high explaining more than 40% of the variability between two variables in both the species.

Therefore, multiple (partial) regression analysis was carried out to see how much every climatic factor contributes to the seasonal activity of the vascular cambium. It was evident from multiple (partial) regression analysis; that monthly mean temperature was statistically significant with the average width of cambial zone ($t \geq +2.72$) and the average width of differentiating xylem zone ($t \geq +3.23$) in *M. champaca* (Table 7.3A). Similarly in *M. oblonga,* monthly mean temperature was statistically significant relationship with the average width of cambial zone ($t \geq +3.33$) and the average width of differentiating xylem zone ($t \geq +4.38$). The average length of fusiform initials ($t \geq +3.30$) and vessel elements ($t \geq +3.29$) showed significant relationship with monthly mean precipitation in *M. oblonga* (Table 7.3B). The monthly mean minimum ambient temperature though, showed statistically significant but inverse relationship with the average width of cambial zone ($t \geq -2.45$) in *M. champaca,* whereas, in *M. oblonga,* monthly mean minimum temperature showed statistically significant inverse relationship with the average width of cambial zone (*t* ≥ -3.54) and average width of differentiating xylem zone (*t* ≥ -3.65). In *M. champaca*, the average length of fusiform initials, xylem fibres and vessel elements did not show any significant (*t*) values with any one of the climatic factors and in *M. oblonga,* the average length of vessel elements did not show any significant relationship with any one of the climatic factors (Table 7.3 A, B).

The correlation coefficient (*r*) and (*t*) values of different anatomical variables such as the average width of cambial zone, average width of differentiating xylem zone, average length of fusiform initials, xylem fibres and vessel elements versus different climatic factors such as monthly mean, mean minimum, mean maximum temperature, monthly mean rainfall and relative humidity in *M. champaca* and *M. oblonga* were presented (Table 7.3A, B). It was evident from correlation and multiple (partial) regression analysis; monthly mean temperature had some effect on the cambial activity and differentiation of xylem elements in both *M. champaca* and *M. oblonga*. The influence of rainfall and relative humidity on both the cambial activity and differentiations of xylem elements was secondary in nature.

Fig. 7.1A Phenology, duration of cambial activity and dormancy in *Michelia champaca* Linn.

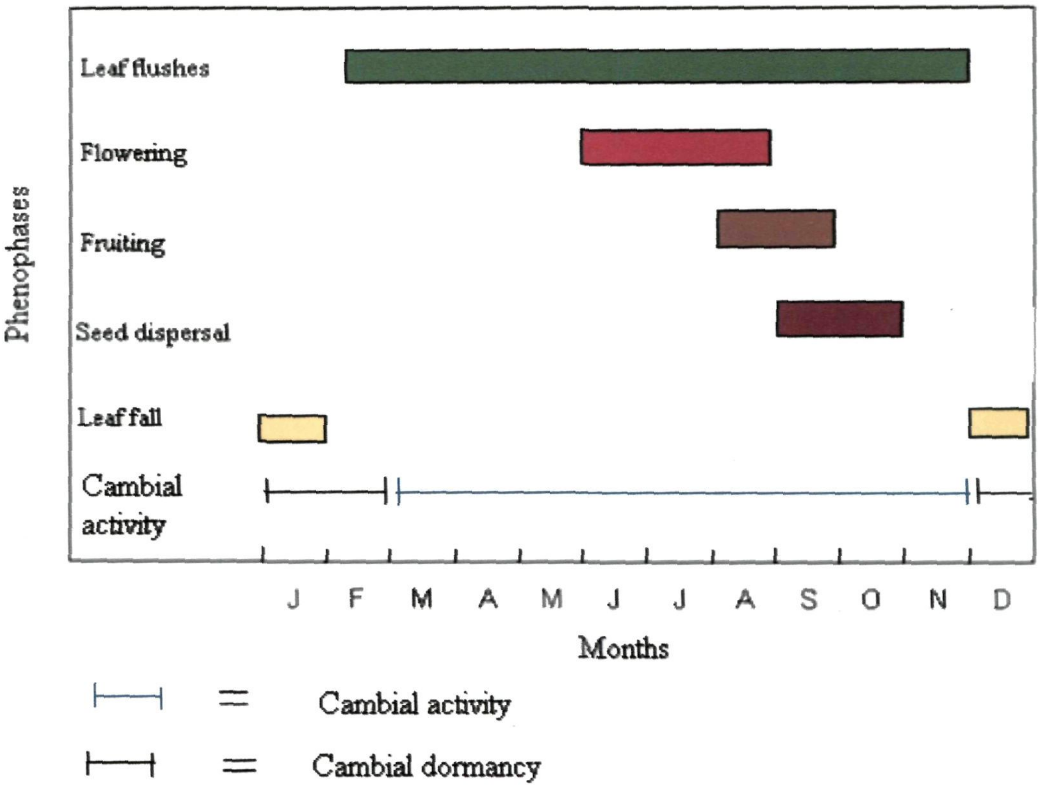


Fig. 7.1B Phenology, duration of cambial activity and dormancy in *Michelia oblonga* Wall. Ex Hk. f.

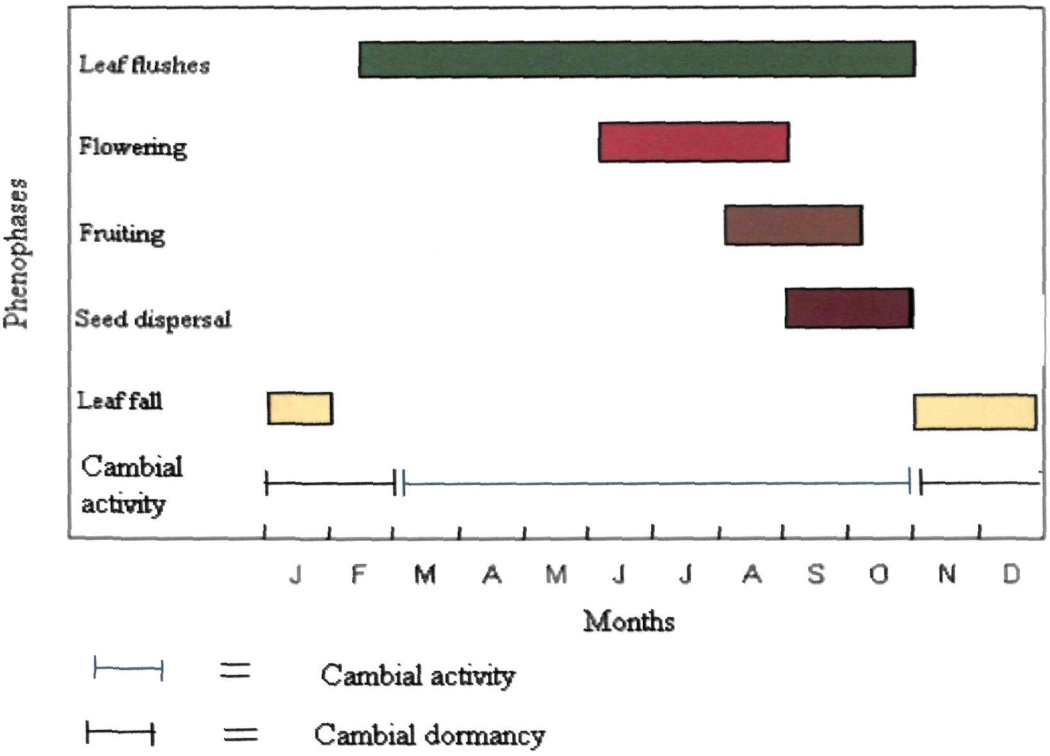


Table 7.1A Average quantitative data of the vascular cambium and its derivatives in *Michelia champaca* Linn.

Months	Numbers of cambial layers.	Average width of cambial zone in μm.	Average width of differentiating xylem zone in μm.	Average length of fusiform initials in μm.	Average length of xylem fibres in μm.	Average length of vessel elements in μm.
February	3-4	47.16 ± 6.42	—	915.31 ± 21.09	1011.36 ± 50.45	529.54 ± 57.89
March	4-5	60.45 ± 8.12	49.59 ± 8.84	944.37 ± 15.62	1172.72 ± 13.48	575.20 ± 22.36
April	5-6	73.00 ± 7.06	107.37 ± 8.36	1034.02 ±19.49	1261.36 ± 45.22	602.27 ± 23.59
May	7-8	93.07 ± 10.52	138.15 ± 15.07	1084.30 ± 23.32	1288.63 ± 51.67	690.91 ± 20.26
June	8-9	101.04 ± 6.90	151.33 ± 13.45	1102.71 ± 19.88	1281.78 ± 56.01	752.27 ± 42.50
July	7-8	98.50 ± 8.25	160.29 ± 14.84	1069.23 ± 15.13	1247.72 ± 57.50	775.01 ± 48.26
August	6-7	89.13 ± 8.78	134.23 ± 13.43	1037.81 ± 14.17	1204.54 ± 40.11	731.81 ± 32.80
September	6-7	87.21 ± 12.73	132.25 ± 14.00	1045.01 ± 26.17	1170.45 ± 53.15	686.36 ± 40.87
October	5-6	64.77 ± 5.34	78.12 ± 10.58	983.00 ± 14.17	1122.79 ± 49.31	634.09 ± 35.83
November	4-5	57.24 ± 6.11	37.10 ± 5.79	930.64 ± 11.83	1086.36 ± 35.99	570.45 ± 43.03
December	3-4	42.27 ± 4.17	—	907.19 ± 21.00	1020.45 ± 51.01	534.09 ± 47.09
January	3-4	40.57 ± 3.49	—	918.43 ± 16.52	1029.54 ± 38.43	531.72 ± 36.20

— = No xylem production
± = Standard deviation

Table 7.1B Average quantitative data of the vascular cambium and its derivatives in *Michelia oblonga* Wall. Ex Hk. f.

Months	Numbers of cambial layers.	Average width of cambial zone in µm.	Average width of differentiating xylem zone in µm.	Average length of fusiform initials in µm.	Average length of xylem fibres in µm.	Average length of vessel elements in µm.
February	3-4	48.03 ±7.40	–	937.01 ± 20.05	1047.31 ±48.02	618.09 ±23.70
March	4-5	56.16 ±8.86	49.41 ±6.06	991.32 ±18.13	1090.81 ±37.53	654.43 ±29.30
April	5-6	72.22 ±7.31	85.36 ±6.63	1057.29 ± 17.91	1163.23 ±30.02	693.18 ±37.23
May	6-7	87.17 ±8.73	106.24 ±8.29	1103.48 ± 25.31	1225.71 ±48.15	688.13 ±34.21
June	7-8	94.32 ±5.86	137.11 ±8.48	1165.08 ± 13.60	1372.56 ±29.81	700.42 ±37.08
July	8-9	100.21 ±5.65	136.39 ±10.61	1184.32 ± 14.27	1406.81 ±31.80	761.36 ±46.05
August	7-8	92.40 ±6.05	128.65 ±7.09	1108.62 ±21.35	1286.36 ±41.60	827.01 ±30.25
September	6-7	85.27 ±5.45	99.45 ±5.53	1062.78 ±994.02	1243.18 ±37.10	740.90 ±22.86
October	4-5	51.21 ±9.18	35.40 ±3.17	994.02 ±11.13	1152.27 ±30.52	695.15 ±31.26
November	3-4	46.26 ±7.11	–	931.52 ±22.70	1097.19 ±35.21	622.07 ±34.36
December	3-4	43.38 ±4.99	–	935.00 ±14.36	1018.33 ±24.19	615.90 ±32.15
January	3-4	42.61 ±3.94	–	928.43 ±24.69	1031.77 ±40.15	618.17 ±33.70

– = No xylem production
± = Standard deviation

Table 7.2A The correlation coefficients (*r*) and (*t*) values of the vascular cambium and its derivatives versus different climatic factors in *Michelia champaca* Linn.

	Monthly mean temperature in°C		Monthly mean maximum temperature in°C		Monthly mean minimum temperature in°C		Monthly mean precipitation in mm		Monthly mean relative humidity in %	
	r	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Average width of cambial zone in µm	0 94*	+2 72*	0 89*	-2 01	0 94*	-2 45*	0 93*	+2 12	0 63*	-0 62
Average length of fusiform initials in µm	0 90*	+1 36	0 85*	-0 19	0 90*	-1 97	0 92*	+2 13	0 55	-1 10
Average width of differentiating xylem zone in µm	0 93*	+3 23*	0 86*	-2 07	0 93*	-2 19	0 87*	+1 77	0 57	-2 10
Average length of xylem fibres in µm	0 85*	+1 29	0 85*	-0 57	0 83*	-1 47	0 80*	+2 03	0 38	+0 41
Average length of vessel elements in µm	0 92*	+1 03	0 86*	-0 19	0 94*	-1 49	0 94*	+1 74	0 74*	+0 01

All *r and *t** values are significant at $p < 0.05$.

Table 7.2B The correlation coefficients (*r*) and (*t*) values of the vascular cambium and its derivatives versus different climatic factors in *Michelia oblonga* Wall. Ex Hk. f.

	Monthly mean temperature in°C		Monthly mean maximum temperature in°C		Monthly mean minimum temperature in°C		Monthly mean precipitation in mm		Monthly mean relative humidity in %	
	r	*t*	*r*	*t*	*r*	*t*	*r*	*t*	*r*	*t*
Average width of cambial zone in µm	0 90*	+3 33*	0 85*	-2 22	0 90*	-3 54*	0 90*	+2 17	0 67*	+0 72
Average length of fusiform initials in µm	0 89*	+1 19	0 84*	-0 32	0 90*	-1 64	0 94*	+3 30*	0 61*	+0 53
Average width of differentiating xylem zone in µm	0 84*	+4 38*	0 76*	-2 19	0 81*	-3 65*	0 83*	+2 21	0 58	+2 16
Average length of xylem fibres in µm	0 90*	+0 89	0 83*	-0 73	0 92*	-0 69	0 97*	+3 29*	0 68*	+0 50
Average length of vessel elements in µm	0 88*	+0 26	0 86*	+0 10	0 88*	-0 20	0 69*	+0 62	0 84*	+2 11

All r^* and t^* values are significant at $p < 0.05$.

Fig. 7.2A Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Michelia champaca* Linn.

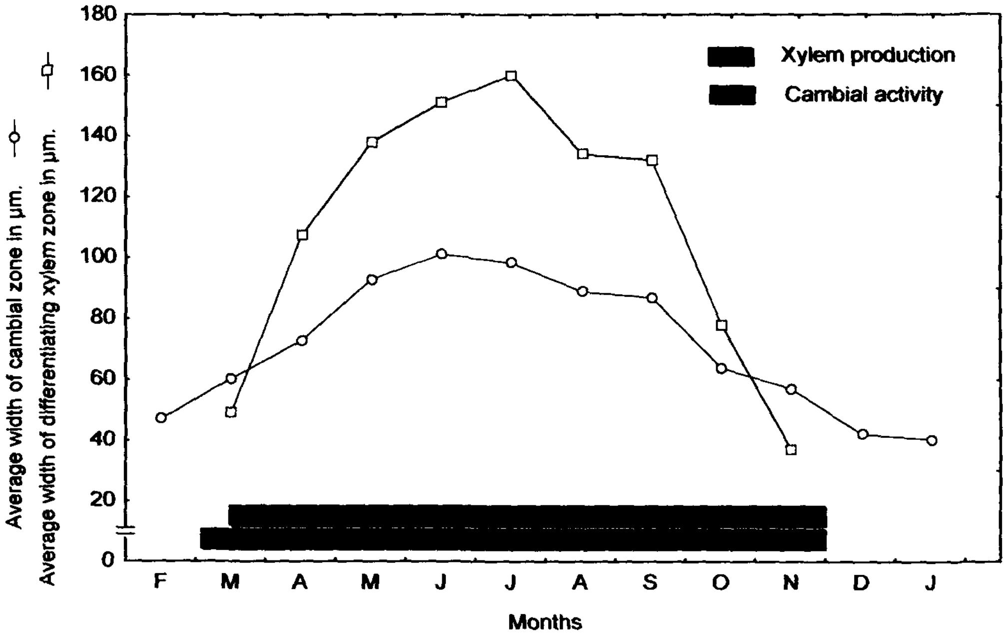


Fig. 7.2B Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Michelia oblonga* Wall. Ex Hk. f.

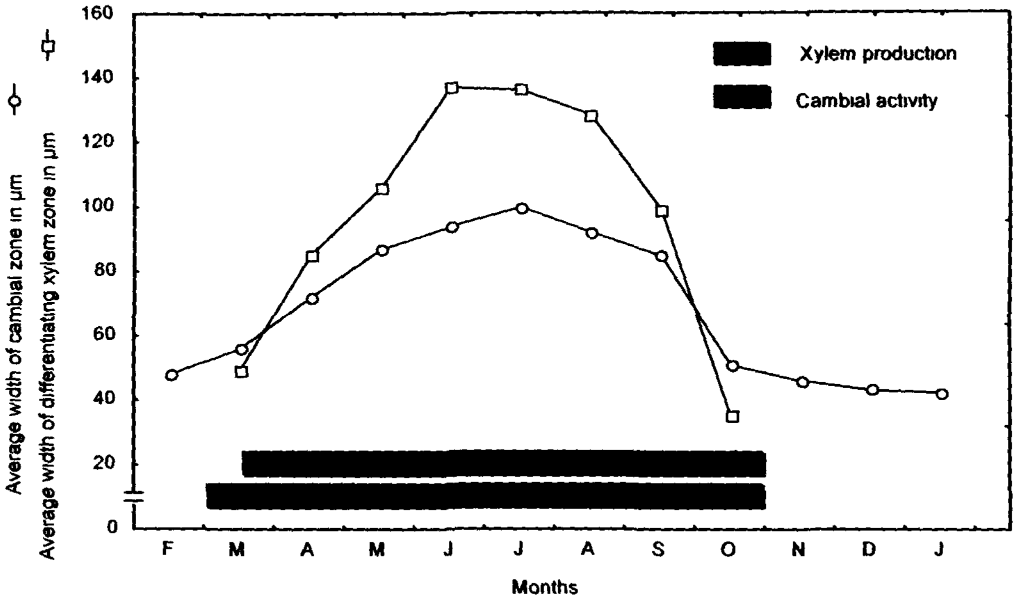


Fig. 7.3A Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Michelia champaca* Linn.

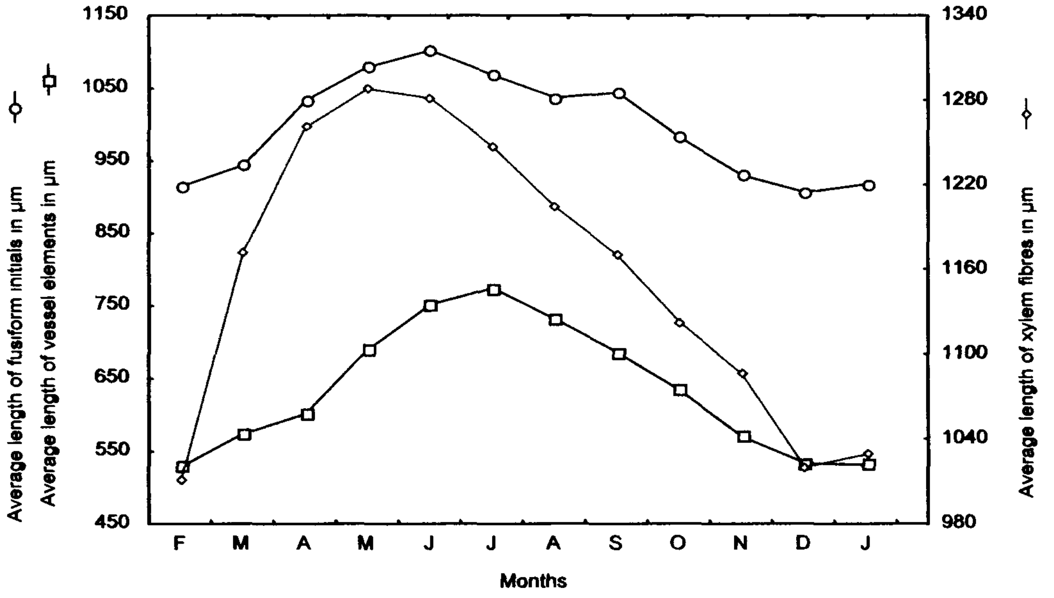


Fig. 7.3B Graphical representation of average quantitative data of the vascular cambium and its derivatives in *Michelia oblonga* Wall. Ex Hk. f.

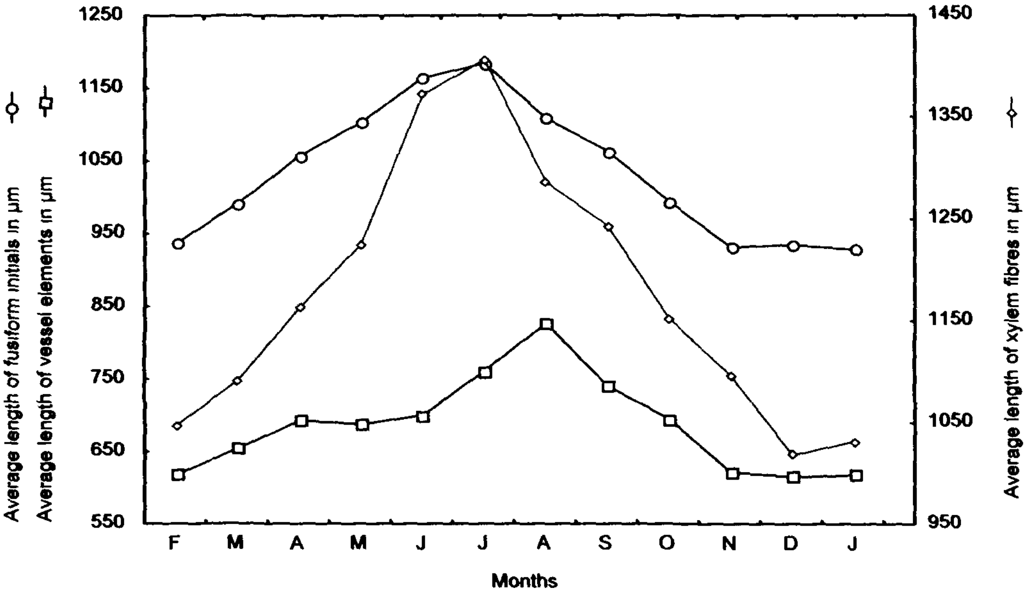
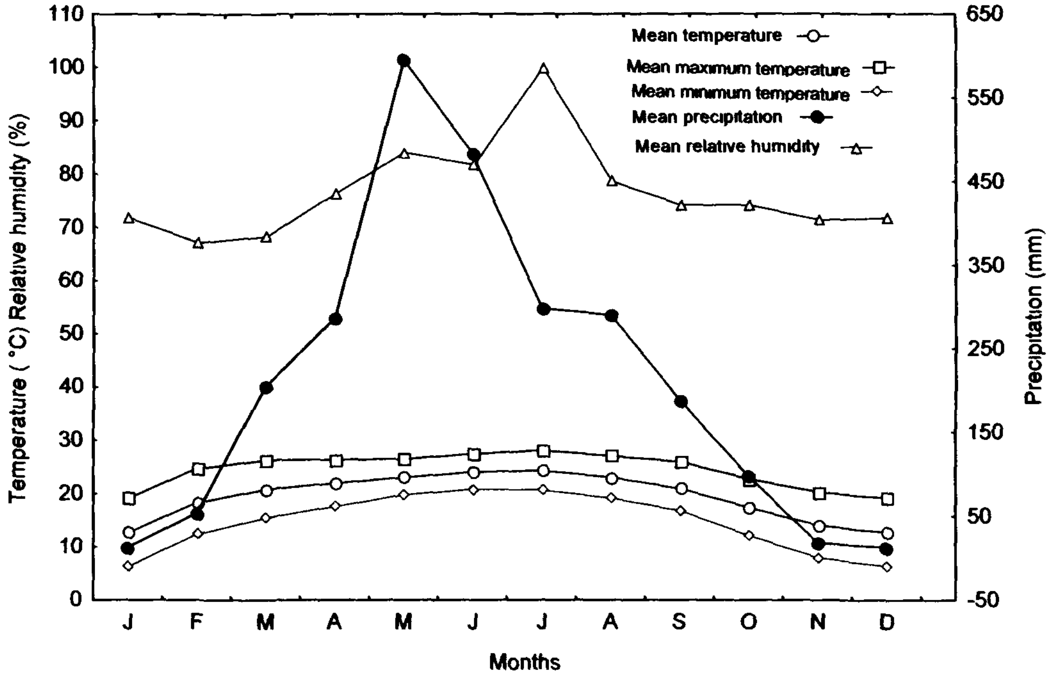
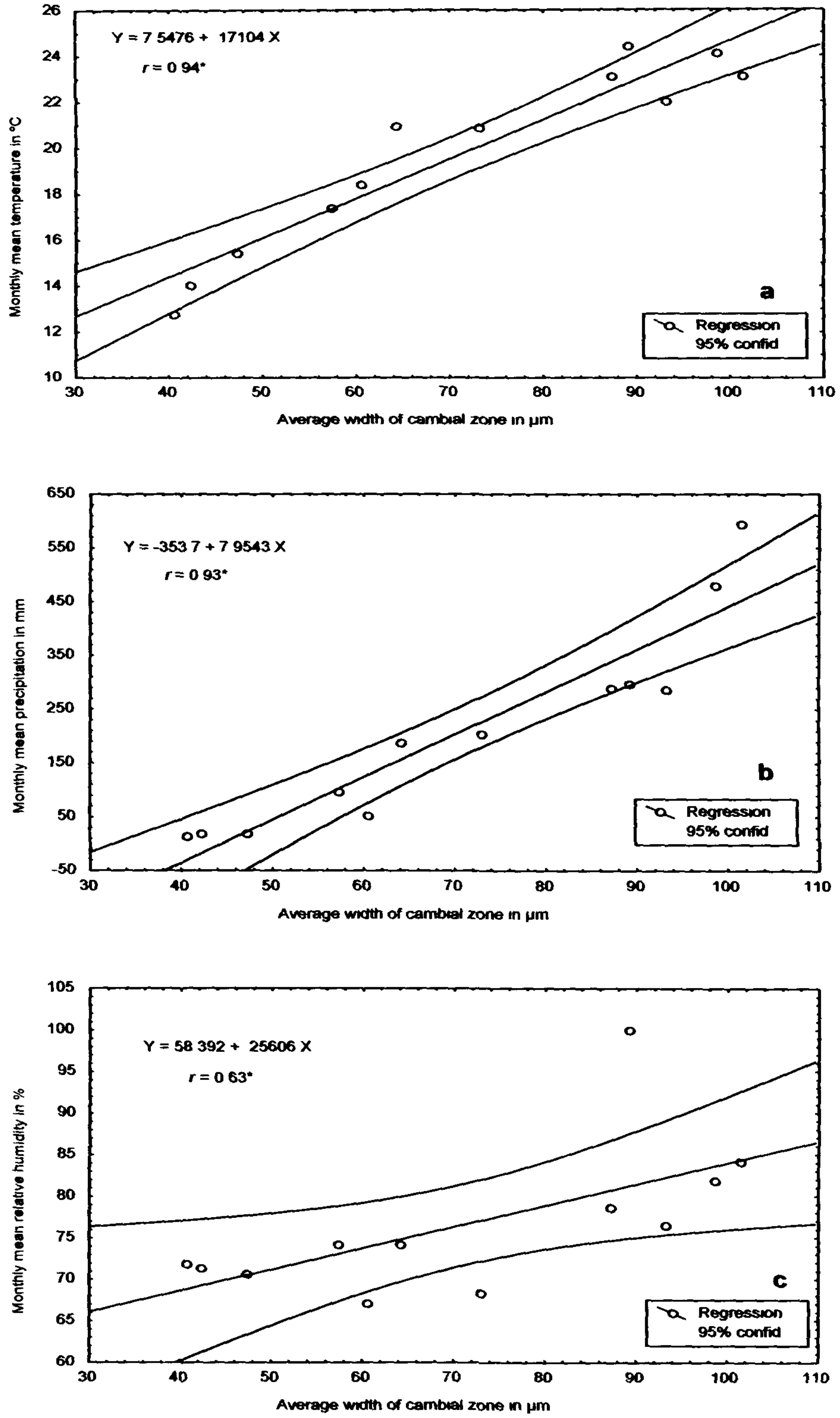


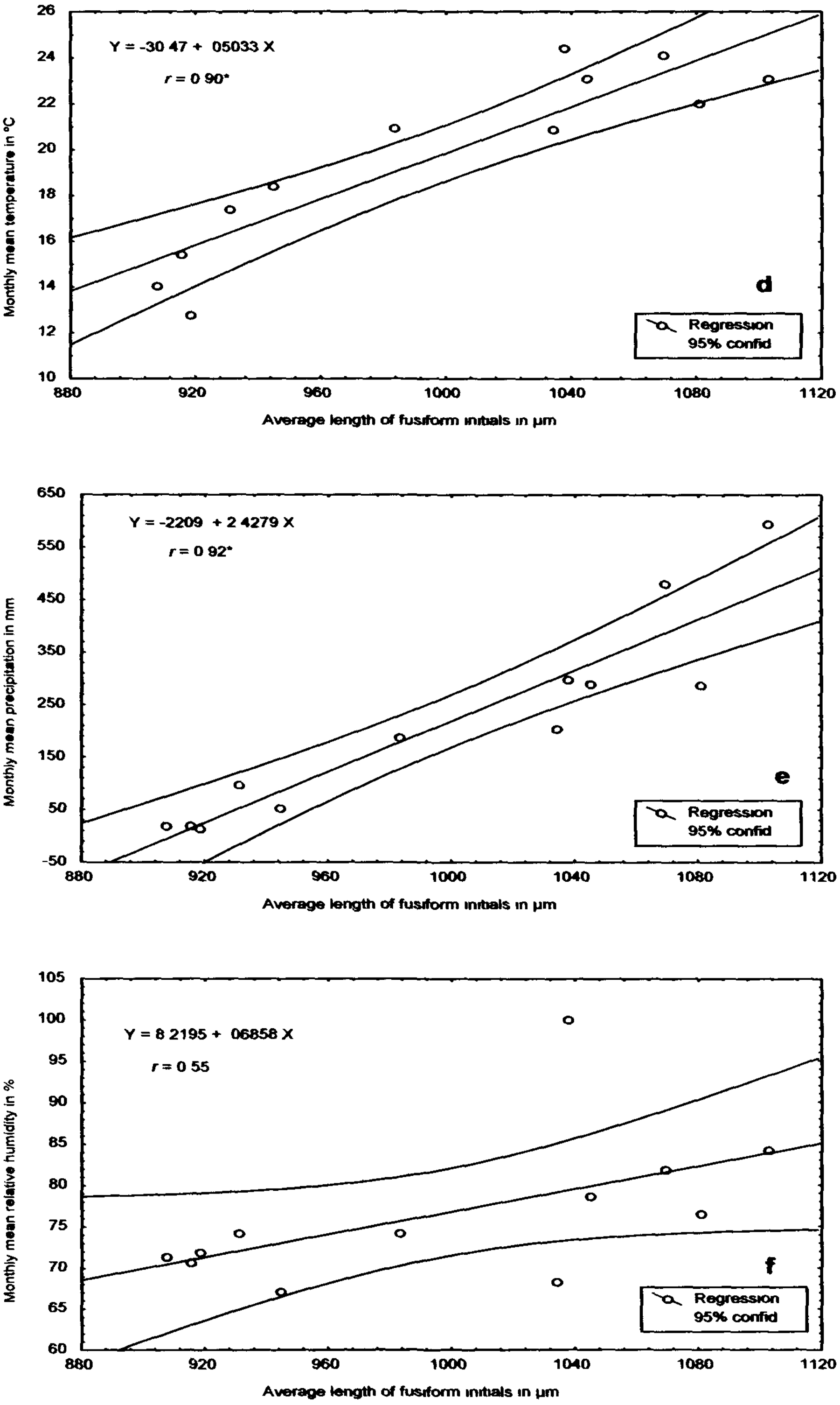
Fig. 7.4 Graphical representation of average climatic data of the years 2002-2003.



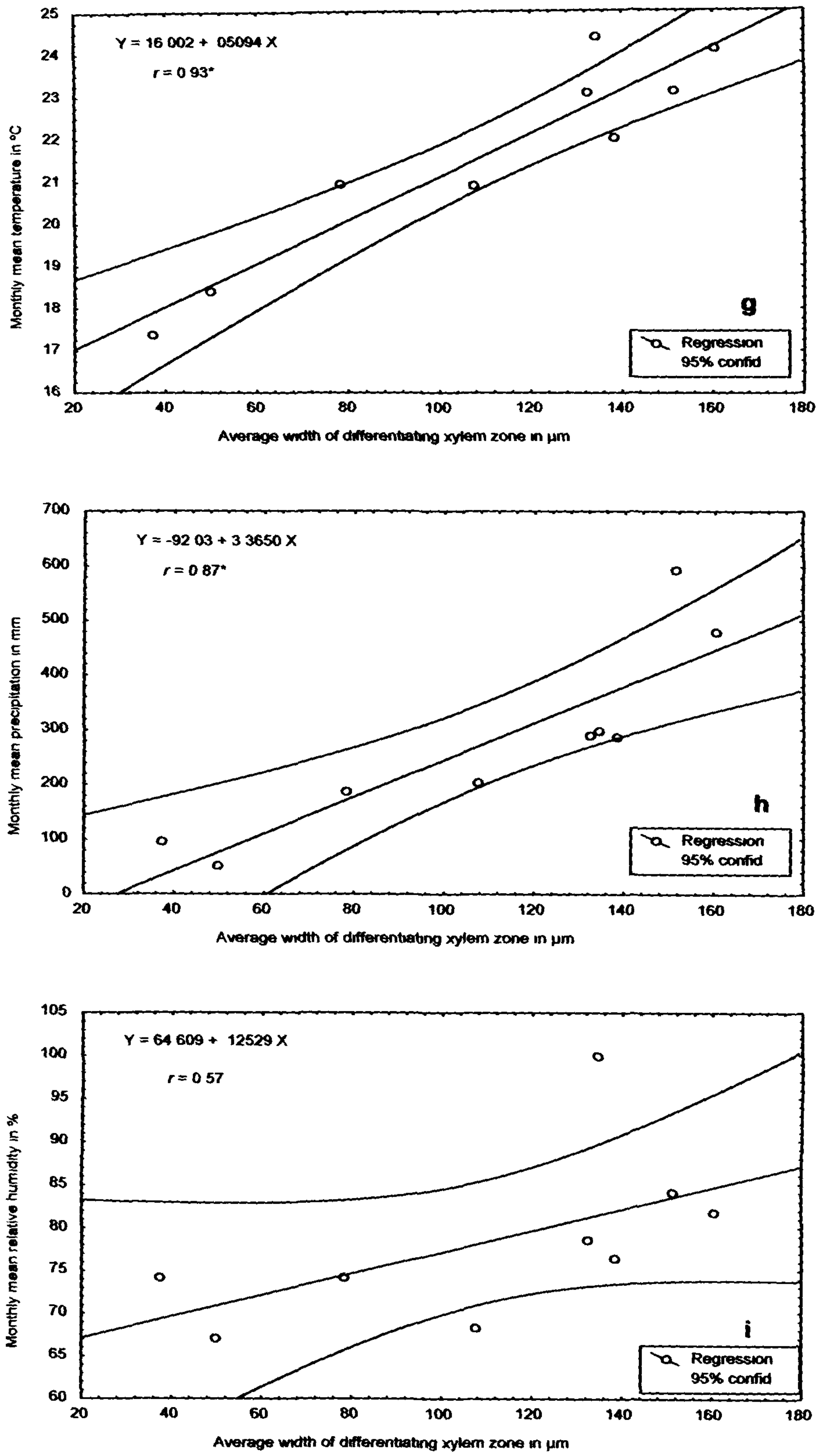
Figs. 7.5A Regression line along with regression equation in *Michelia champaca* Linn. All *r values are significant at $p < 0.05$.



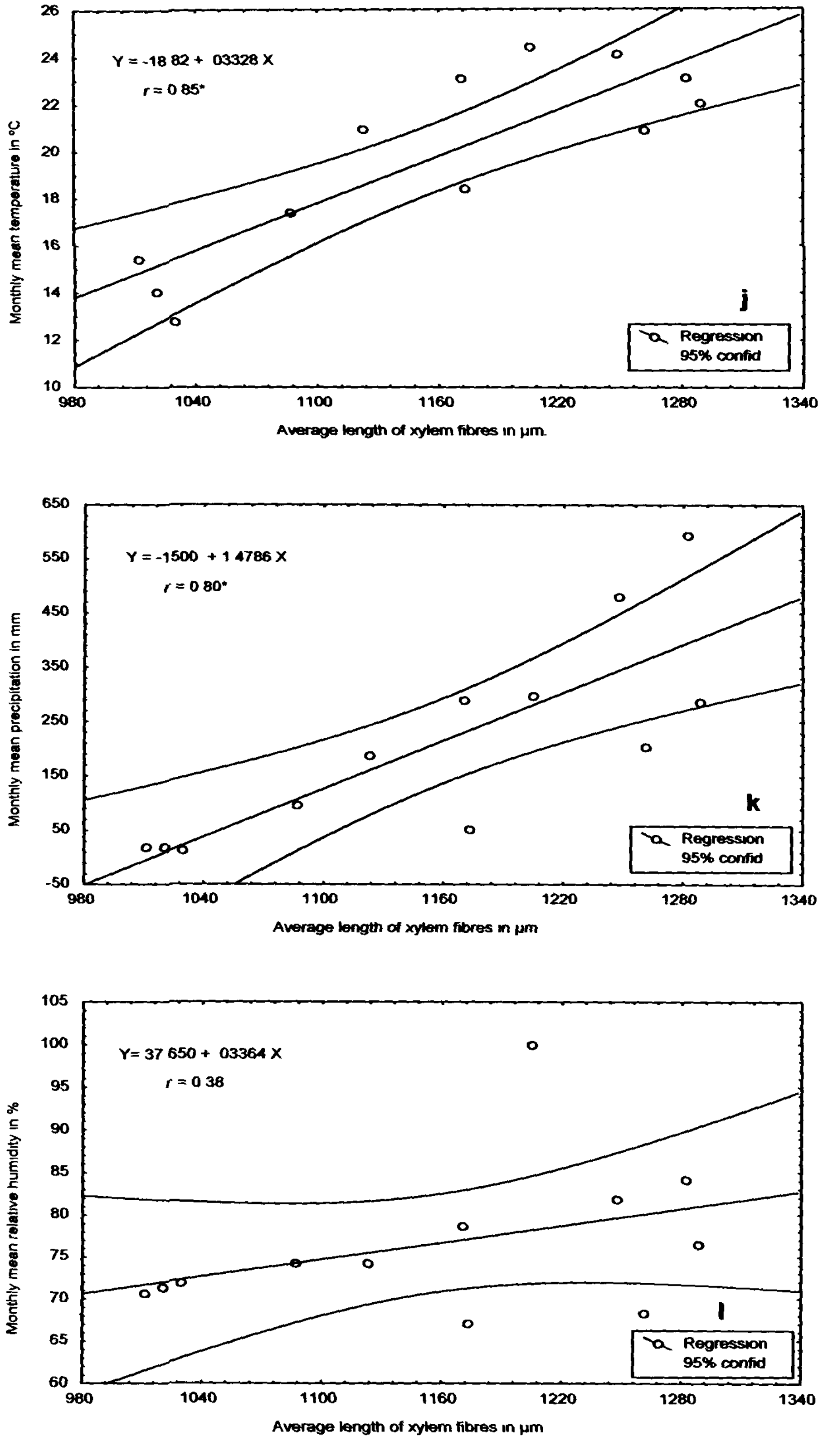
Figs. 7.5A Regression line along with regression equation in *Michelia champaca* Linn. All *r values are significant at $p < 0.05$.



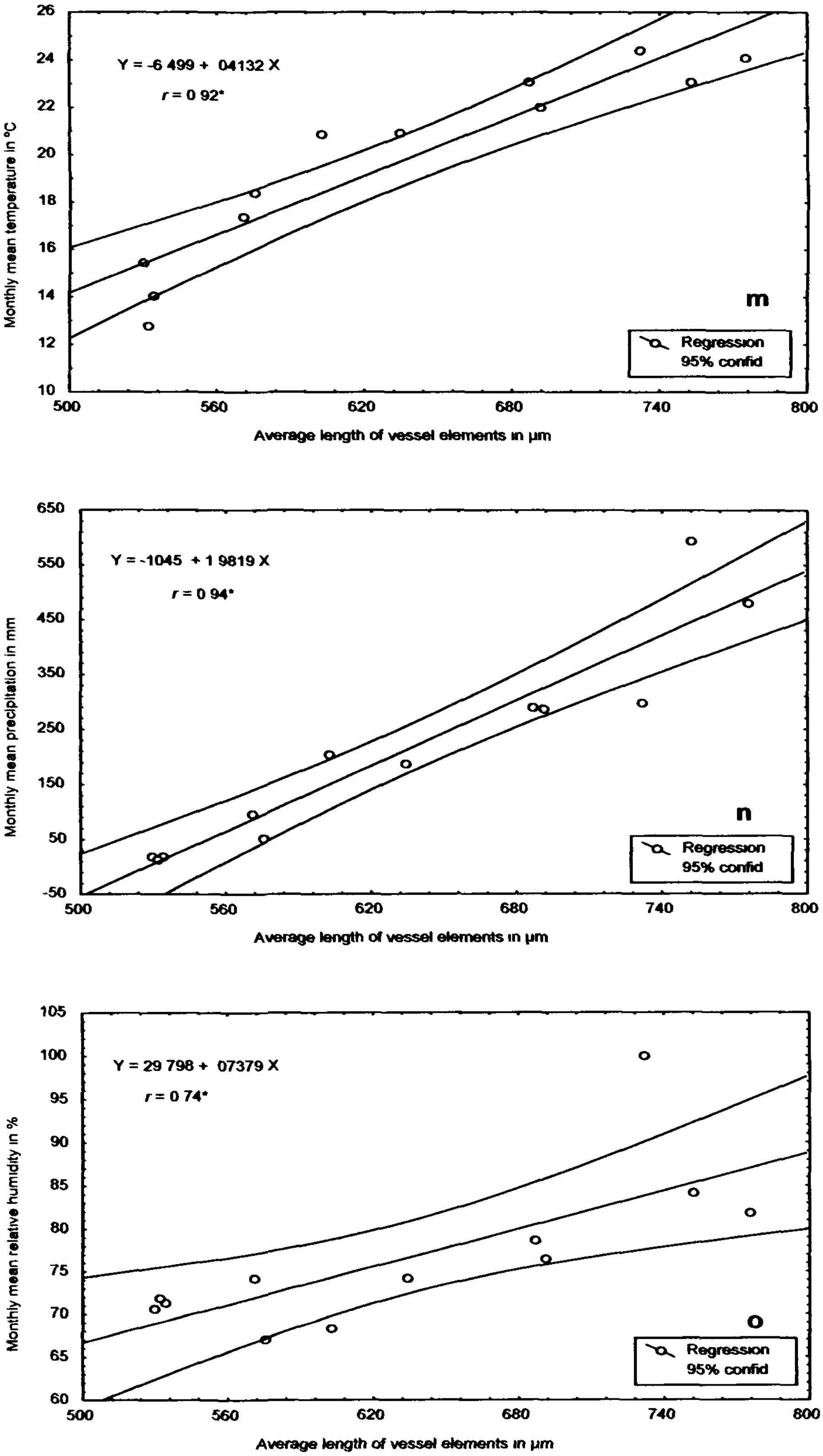
Figs. 7.5A Regression line along with regression equation in *Michelia champaca* Linn. All *r values are significant at $p < 0.05$.



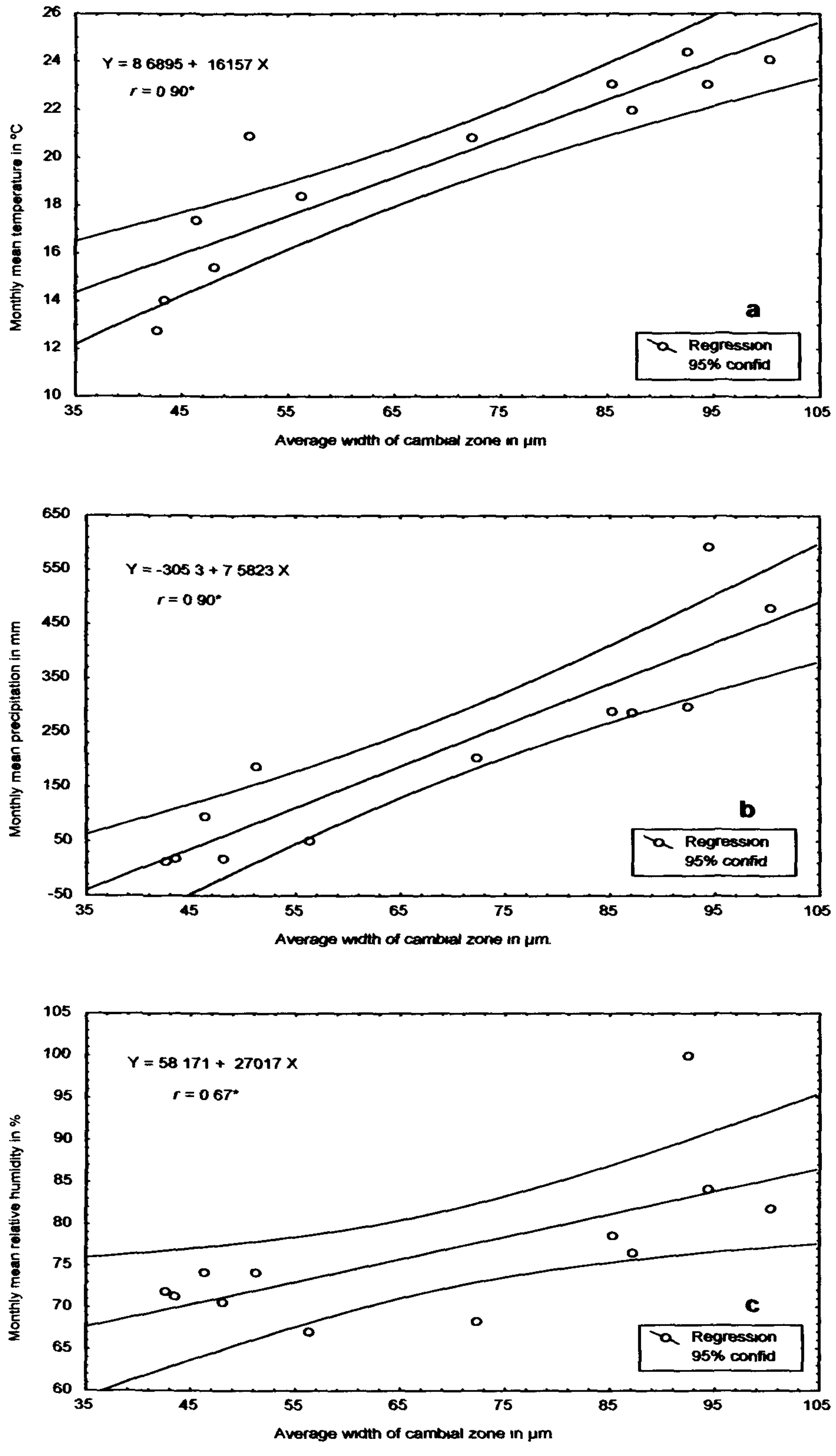
Figs. 7.5A Regression line along with regression equation in *Michelia champaca* Linn. All *r values are significant at $p < 0.05$.



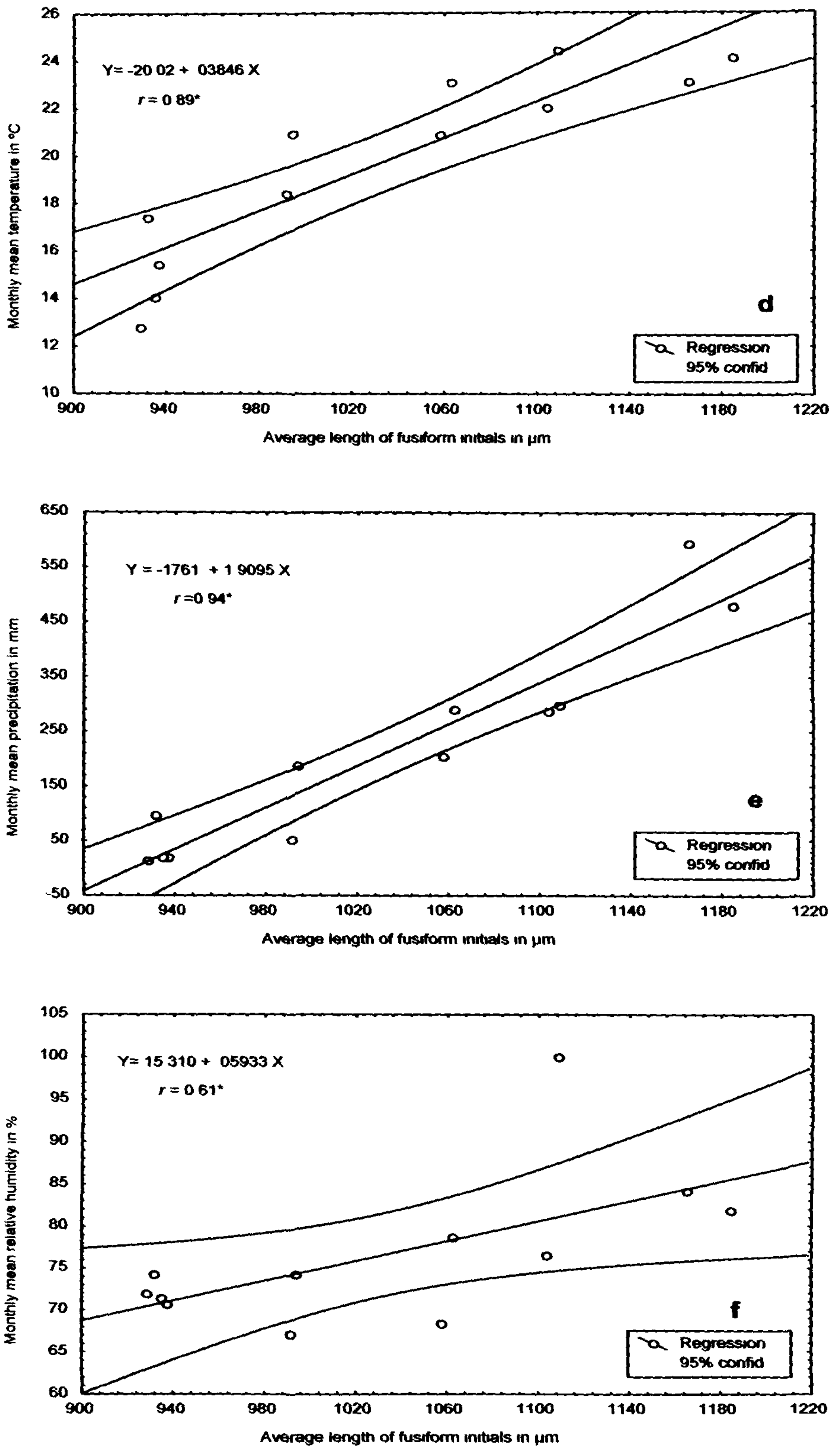
Figs. 7.5A Regression line along with regression equation in *Michelia champaca* Linn. All *r values are significant at $p < 0.05$.



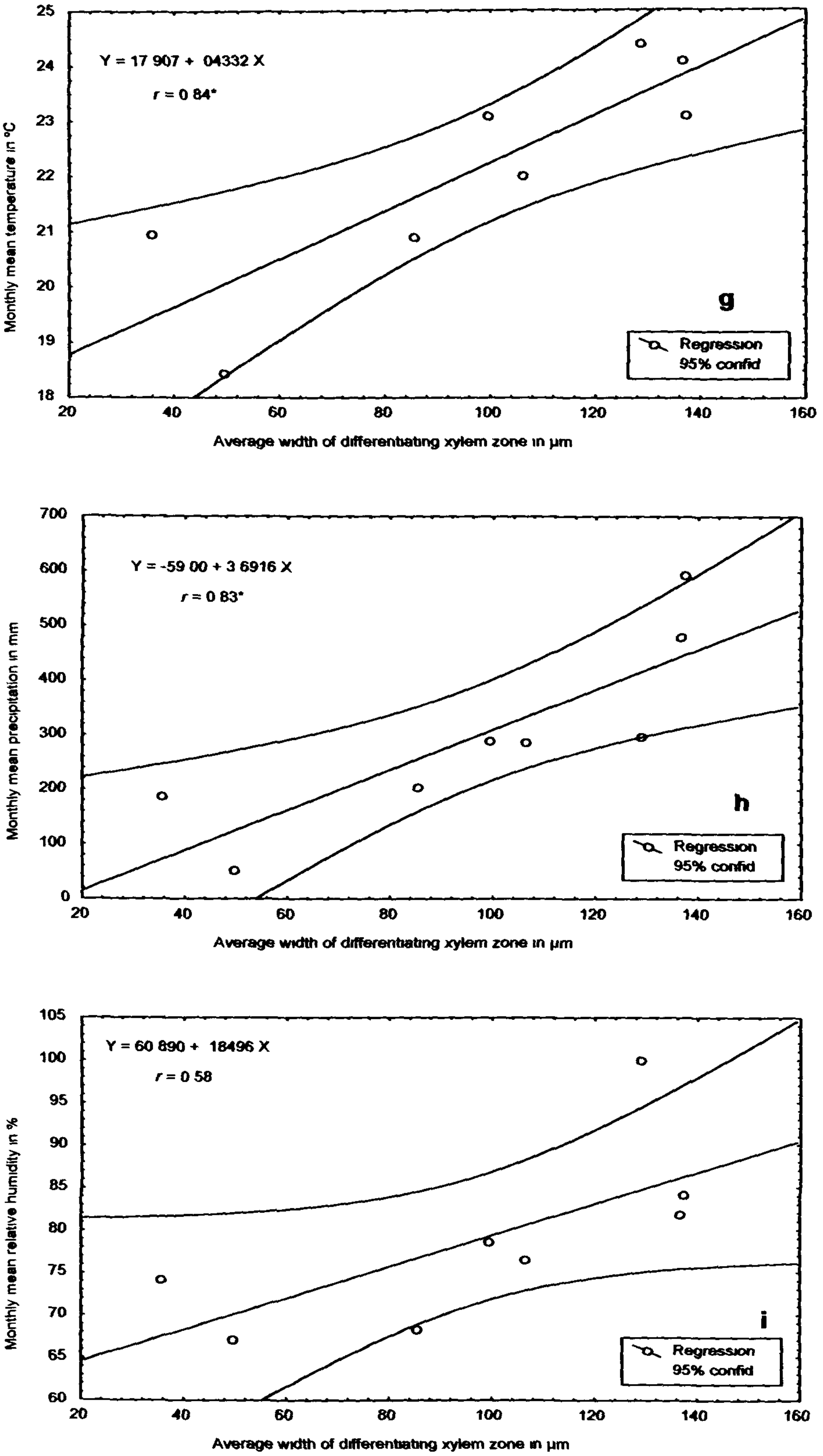
Figs. 7.5B Regression line along with regression equation in *Michelia oblonga* Wall. Ex Hk. f. All *r values are significant at $p < 0.05$.



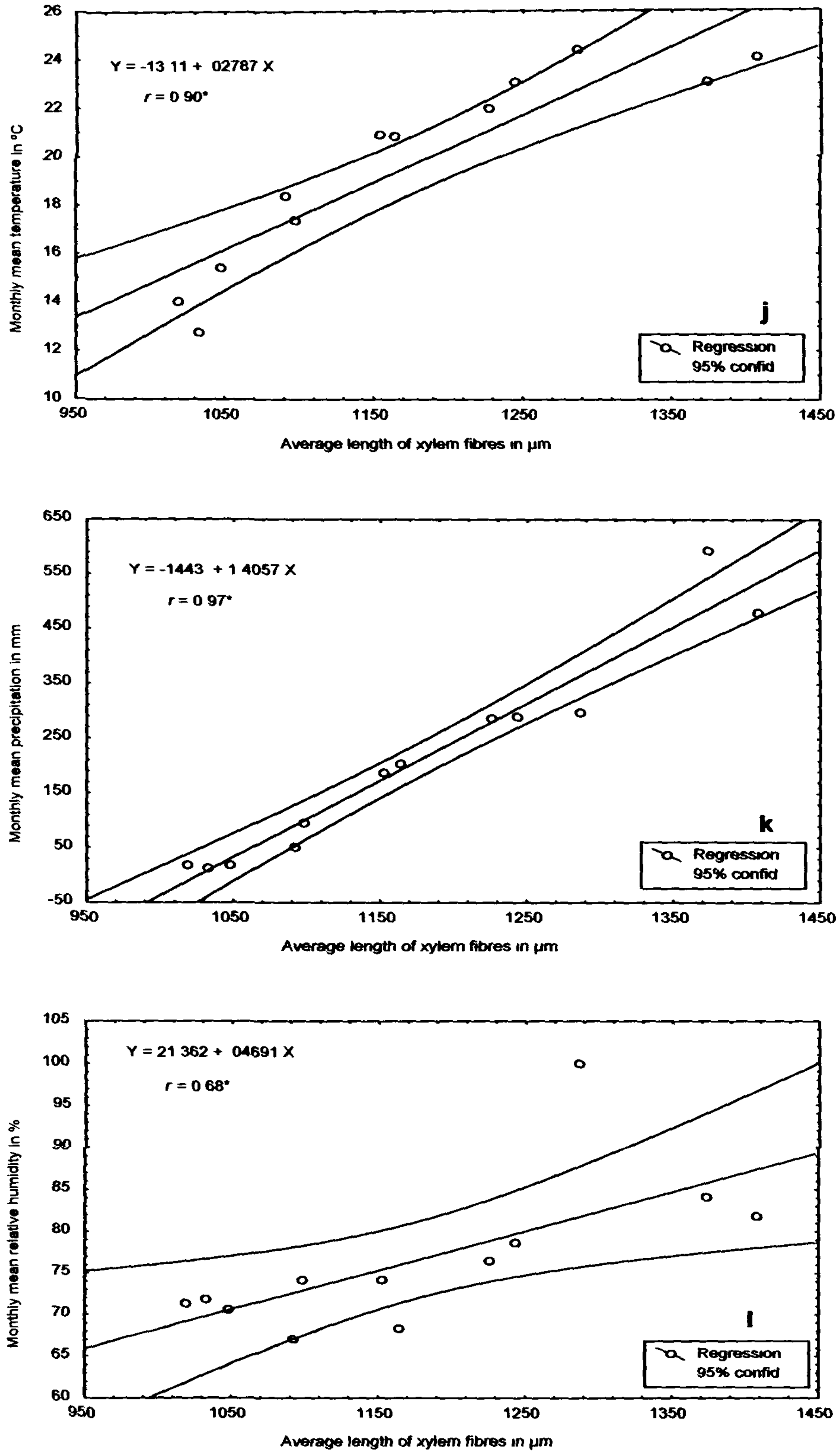
Figs. 7.5B Regression line along with regression equation in *Michelia oblonga* Wall. Ex Hk. f. All *r values are significant at $p < 0.05$.



Figs. 7.5B Regression line along with regression equation in *Michelia oblonga* Wall. Ex Hk. f. All **r* values are significant at $p < 0.05$.



Figs. 7.5B Regression line along with regression equation in *Michelia oblonga* Wall. Ex Hk. f. All *r values are significant at $p < 0.05$.



Figs. 7.5B Regression line along with regression equation in *Michelia oblonga* Wall. Ex Hk. f. All **r* values are significant at *p* < 0.05.

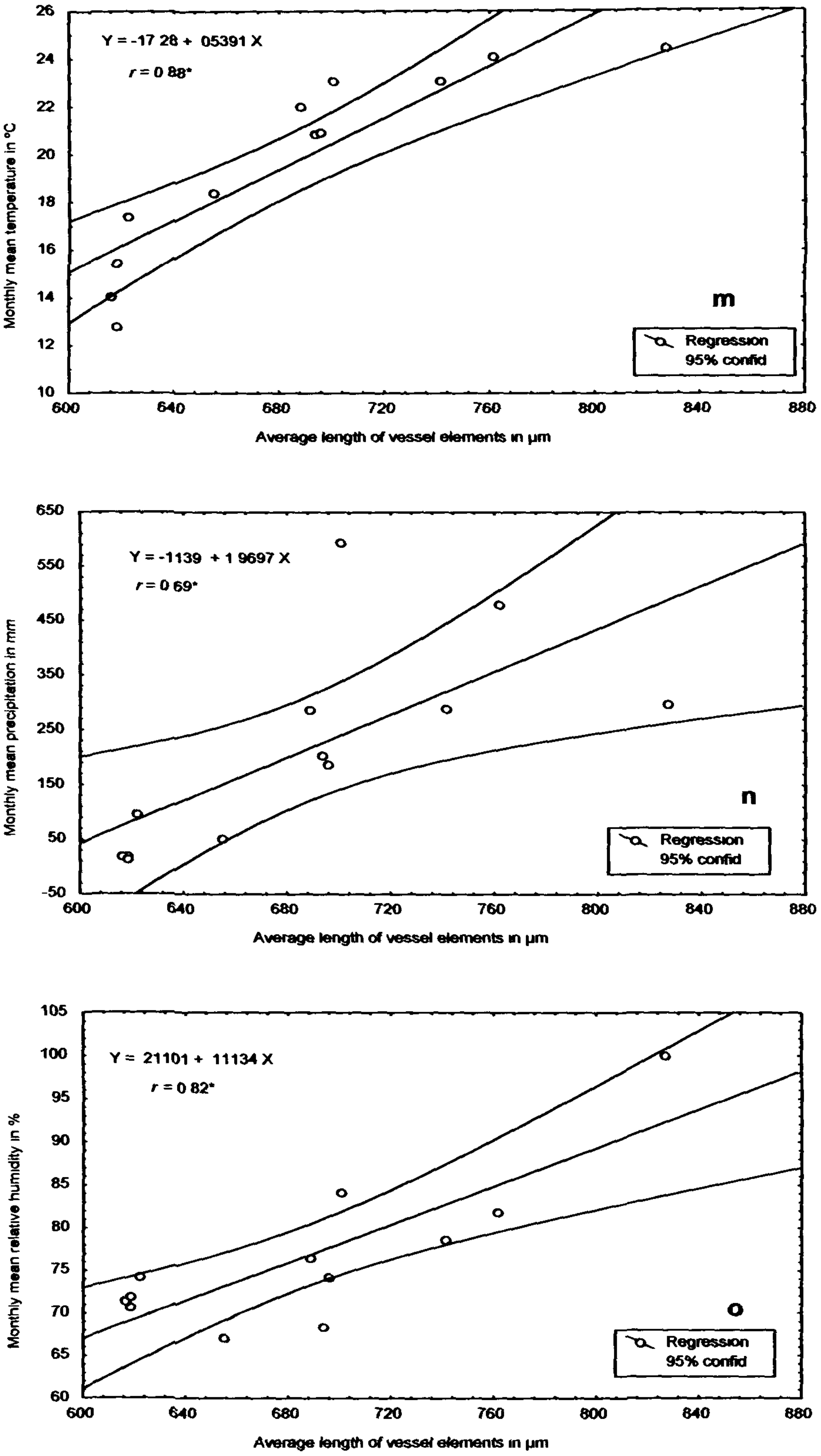


Plate- 7.1

a Tangential longitudinal sections (TLS) of active cambium with fusiform (Fi) and ray (Ri) initials. The beads are not distinct. Note the multinucleate condition of fusiform initials. Rays composed of multiseriate (Mr), biseriate (Br) and uniseriate (Ur) types.

b (TLS) of dormant cambium. The beads are distinct. Note the multinucleate condition of fusiform initials in **(a)** and **(b)**. Rays composed of multiseriate (Mr), biseriate (Br) and Uniseriate (Ur) types.

c (TLS) of active cambium showing fusiform (Fi) and ray (Ri) initials. The beaded appearance is not prominent. Rays composed of multiseriate (Mr), biseriate (Br) and uniseriate (Ur) types. Note the multinucleate condition.

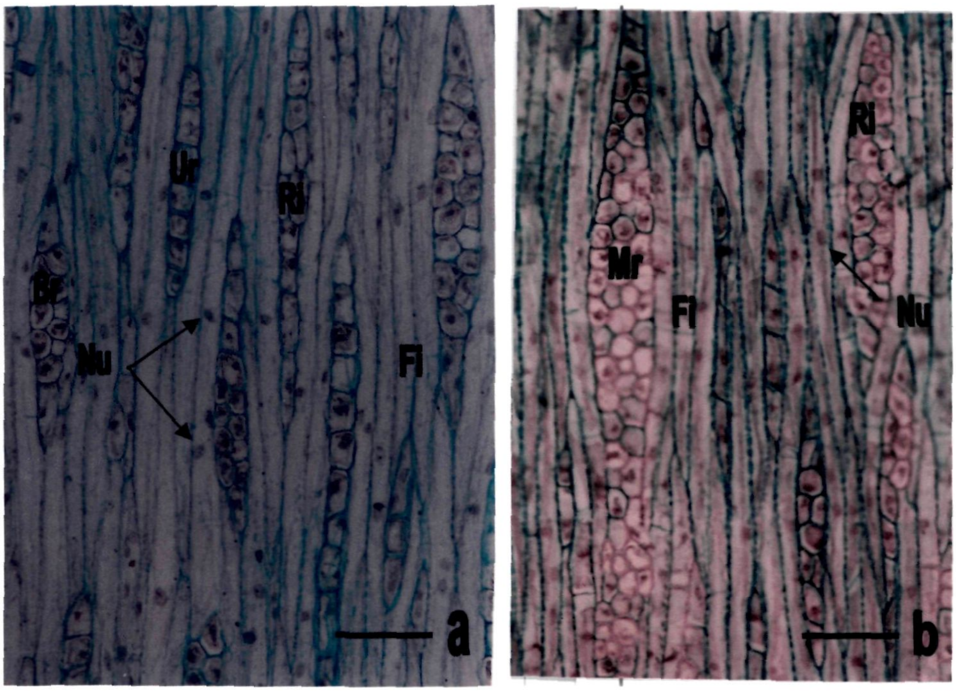
d (TLS) dormant cambium. The beaded cell wall is distinct. Rays composed of multiseriate (Mr), biseriate (Br) and uniseriate (Ur) types.

(Bar: **a** = 145µm; **b** = 120µm; **c**= 160µm; **d** = 125µm)

Plate- 7.1

Michelia champaca Linn. and ***Michelia oblonga*** Wall. Ex Hk. f.

Tangential longitudinal sections of cambium of *M. champaca*
Active Dormant



Tangential longitudinal sections of vascular cambium of *M. oblonga*
Active Dormant

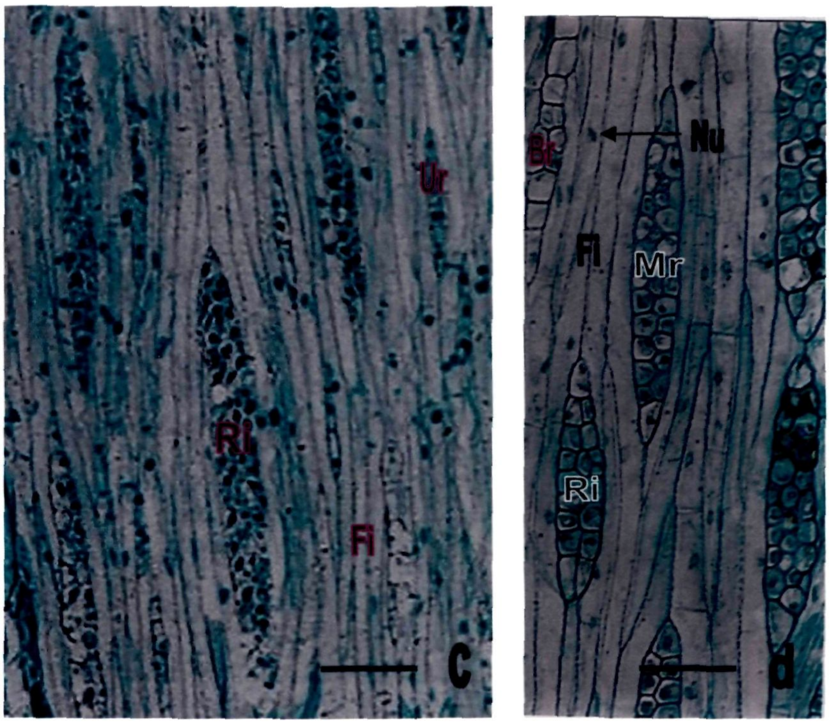


Plate- 7.2

a Transverse section of the reactivation cambium during March showing radial swelling (Rs) and periclinal division (Pd) in the month of March.

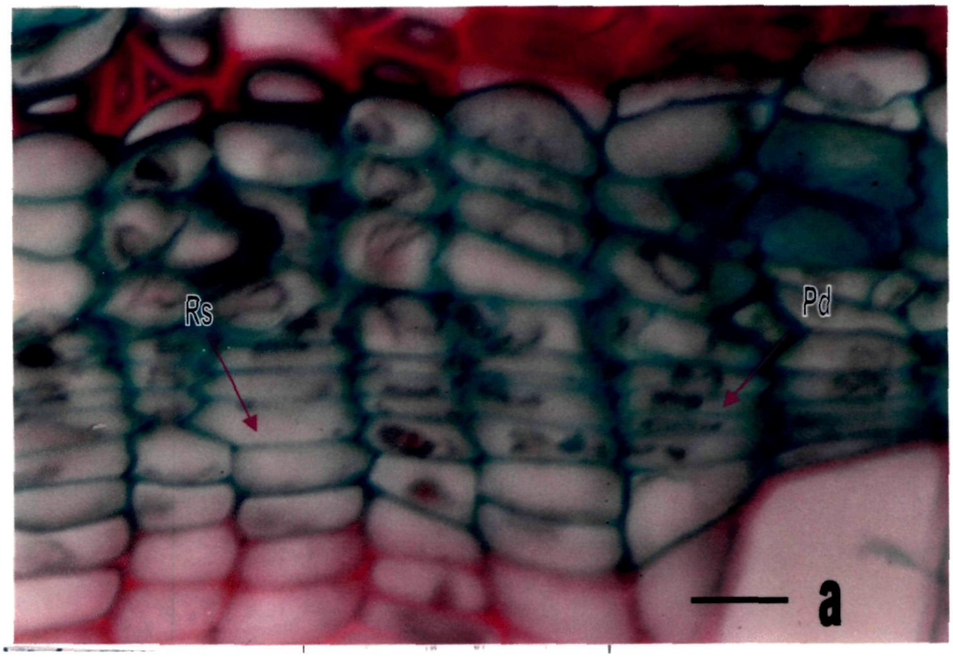
a_1, a_2 Periclinal division in metaphase stage (M)in the fusiform initial.

(Bar: **a** = 40µm; **a_1** = 25µm; **a_2** = 20µm)

Plate- 7.2

Michelia champaca Linn.

Vascular cambium at the time of reactivation



Periclinal division in Metaphase stage

Enlarged view

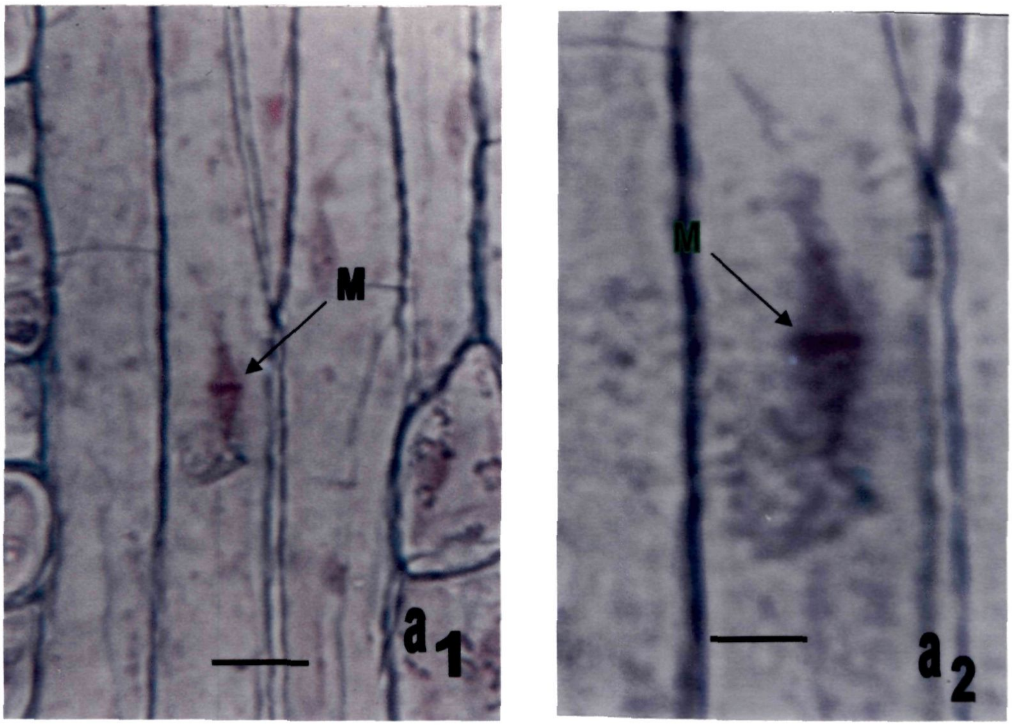


Plate- 7.3

a TS of the active cambium. The cambial (white arrow) consists of 8-9 layers surrounded by differentiating xylem (Dx) and differentiating vessel element (Dv).

b TS of the active cambium showing 8-9 layers in the cambial zone (white arrow). Note differentiating xylem (Dx) and differentiating vessel element (Dv).

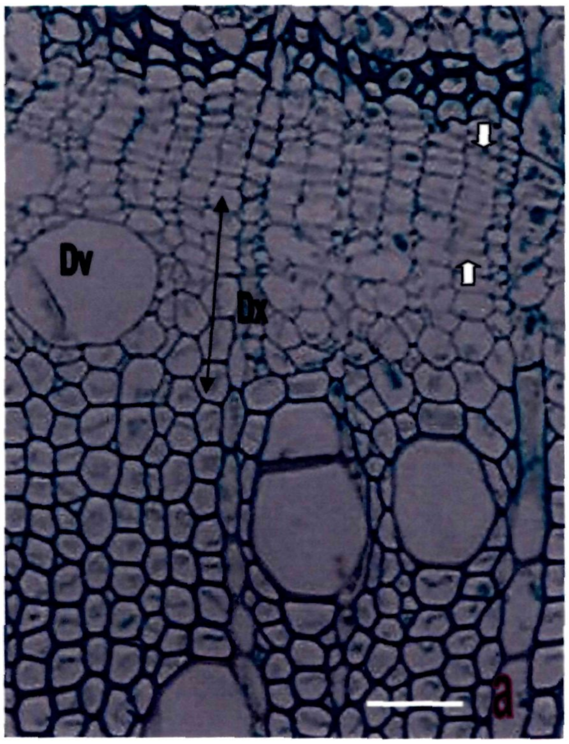
c Enlarged view of (**b**).

(Bar: **a** = 80µm; **b** = 120µm; **c**= 24µm)

Plate- 7.3

Michelia champaca Linn. and ***Michelia oblonga*** Wall. Ex Hk. f.

Transverse section of active vascular cambium of *M. champaca*



Transverse sections of active vascular cambium of *M. oblonga*
Enlarged view

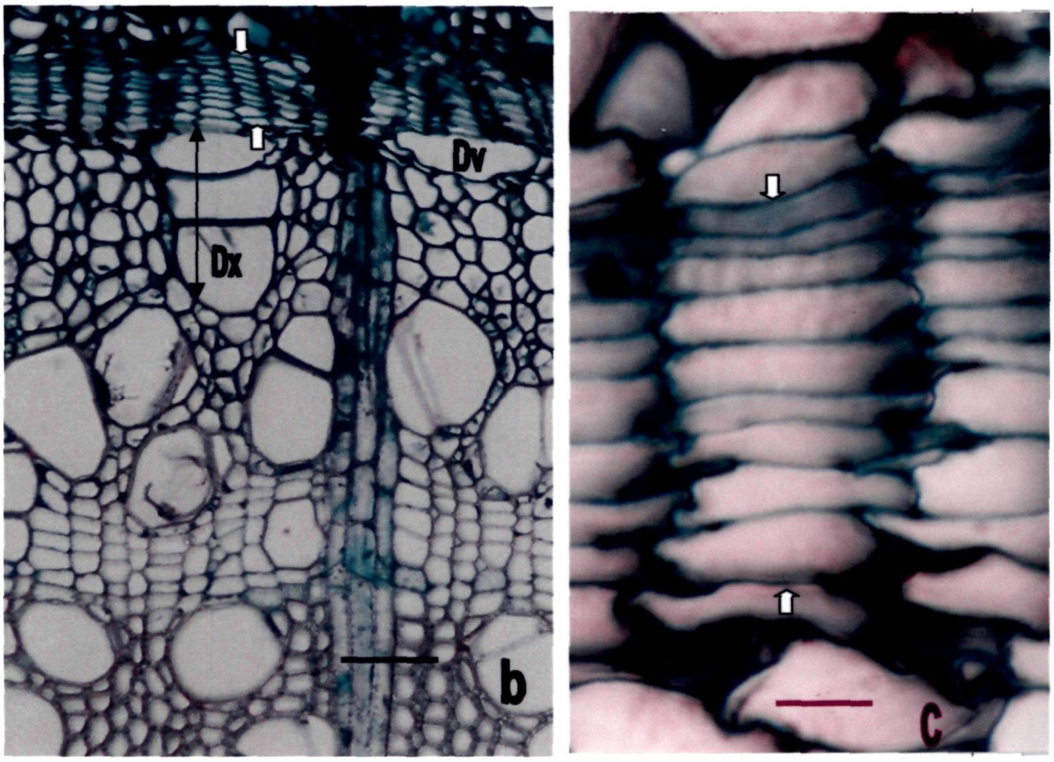


Plate-7.4

a TS of dormant vascular cambium and cambial zone (white arrow) consist of 3-4 layers. Starch grain (S) in xylem rays and parenchyma.

a_1 TLS of cambium showing septate fusiform initials with multinucleate condition towards the end of growth season.

a_2 Macerated septate xylem fibre (Sf).

a_3 Periclinal division with phragmoplast (white arrow) and daughter nuclei (Dn) in the active fusiform initial.

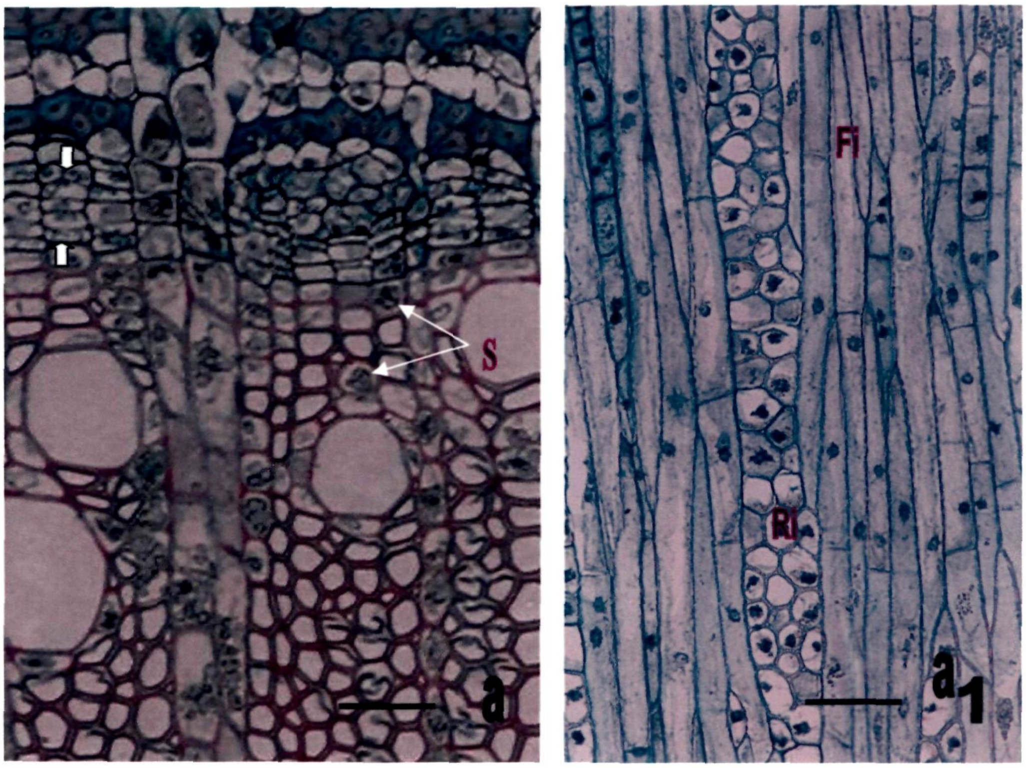
(Bar: **a** = 50µm; **a_1** = 108µm; **a_2**= 125µm; **a_3** = 120µm)

Plate- 7.4

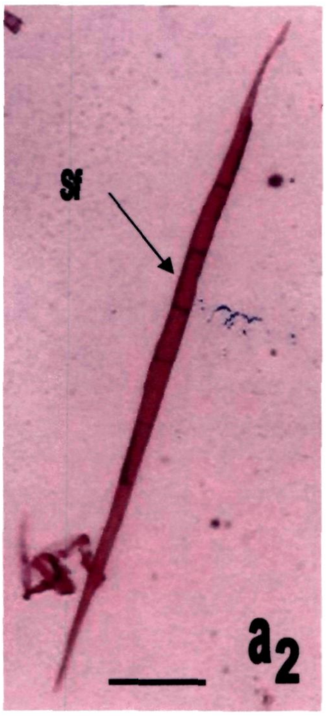
M. champaca Linn.

Dormant vascular cambium

Transverse section   Tangential longitudinal section



Septate xylem fibre



Periclinal division

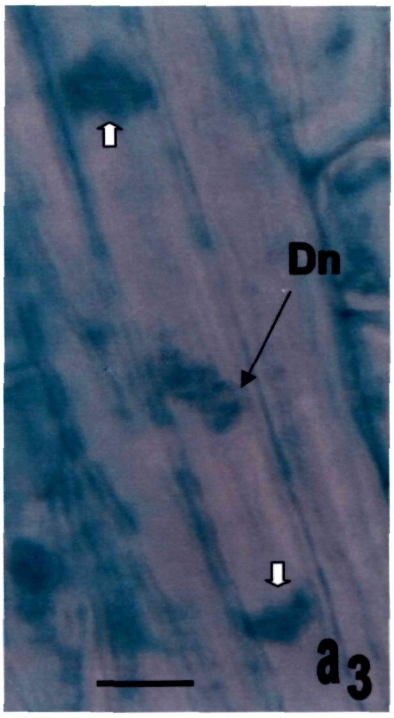


Plate- 7.5

a TS of dormant vascular cambium. The cambial zone (white arrow) consists of 3-4 layers. Starch grain (S) in xylem rays and parenchyma and on the phloem side..

b Macerated vessel elements produce during active period. Scalariform perforation plate (Sp) with 6 bars in *M. champaca.*

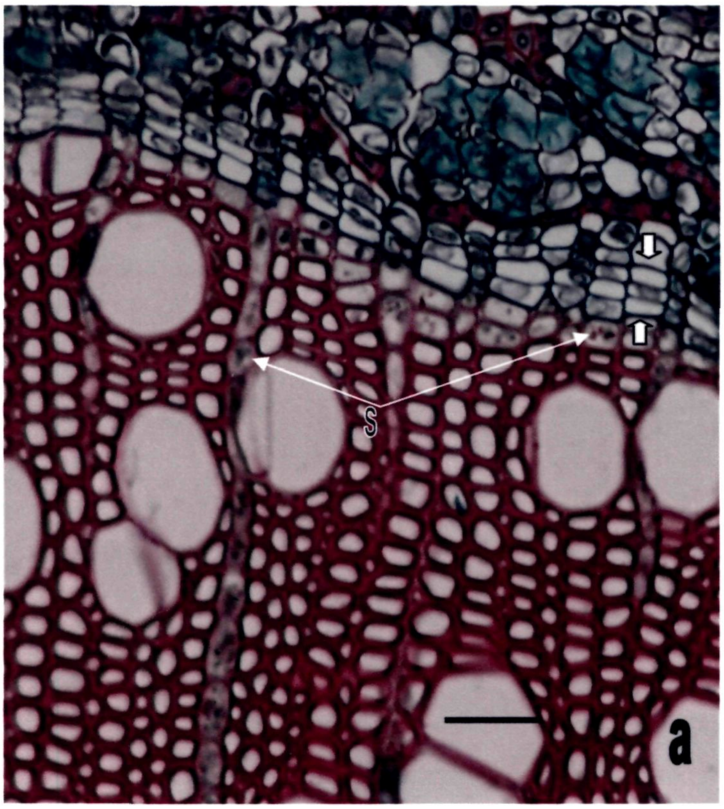
c Macerated late wood vessel elements showing scalariform perforation plate with 3-4 bars in the late wood elements in *M. champaca.*

(Bar: **a** = 65µm; **b** = 50µm; **c**= 75µm)

Plate- 7.5

M. champaca Linn. and ***M. oblonga*** Wall. Ex Hk. f.

Transverse section of dormant cambium of *M. oblonga*



Macerated vessel elements of *M. champaca*

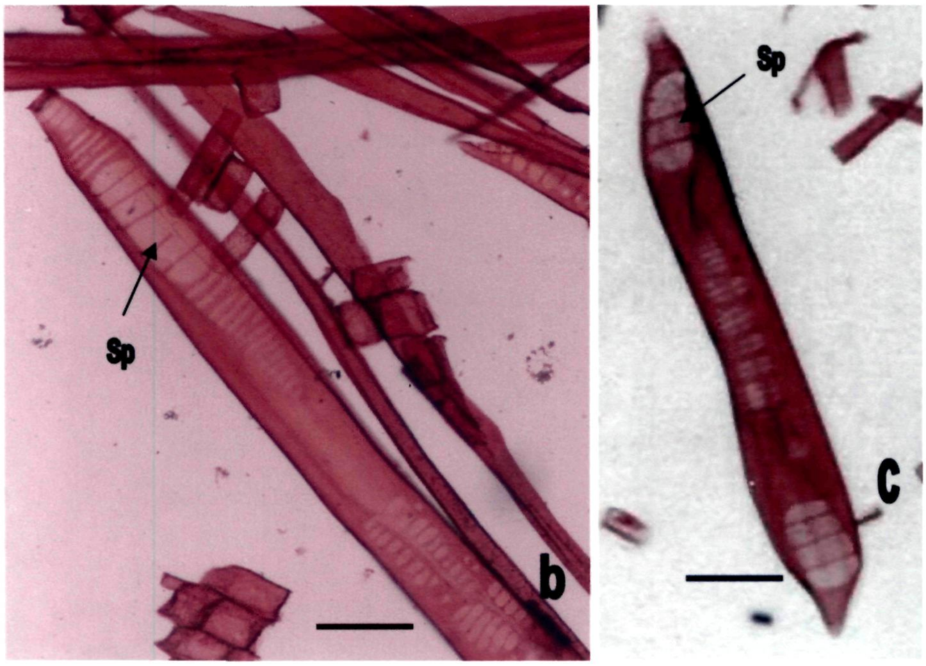


Plate-7.6

a Macerated vessel elements produced during active period showing scalariform perforation plate (Sp) with 6 bars in *M. oblonga*.

b Macerated late wood vessel elements showing scalariform perforation plate with 3-4 bars. Note the lateral wall pittings are also scalariform type in *M. oblonga* (**a**) and (**b**).

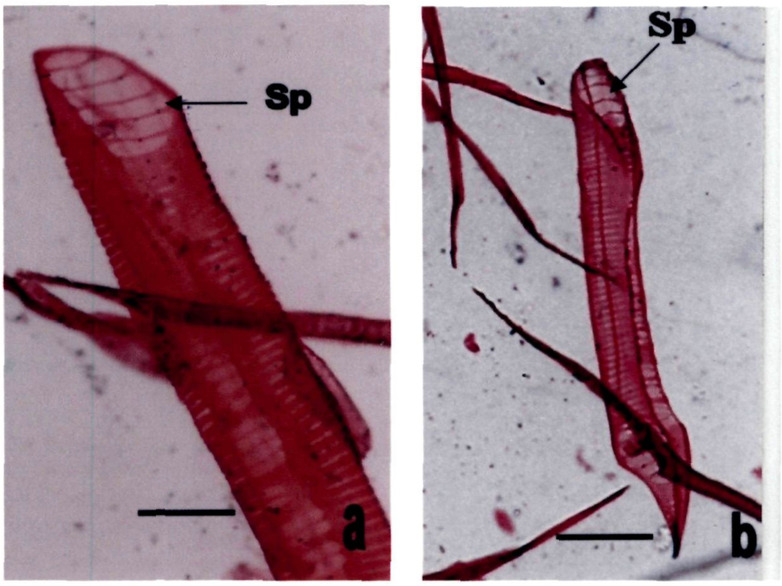
c, d TS of wood in *M. champaca* and *M.oblonga* respectively. The growth rings (white arrow) are distinct in (**c**) and (**d**), with initial parenchyma. Early wood (Ew), late wood (Lw), xylem fibres (Xf). Vessel elements (Ve) are arranged mostly solitary or in radial multiple.

(Bar: **a** = 55µm; **b** = 90µm; **c, d** = 45µm).

Plate- 7.6

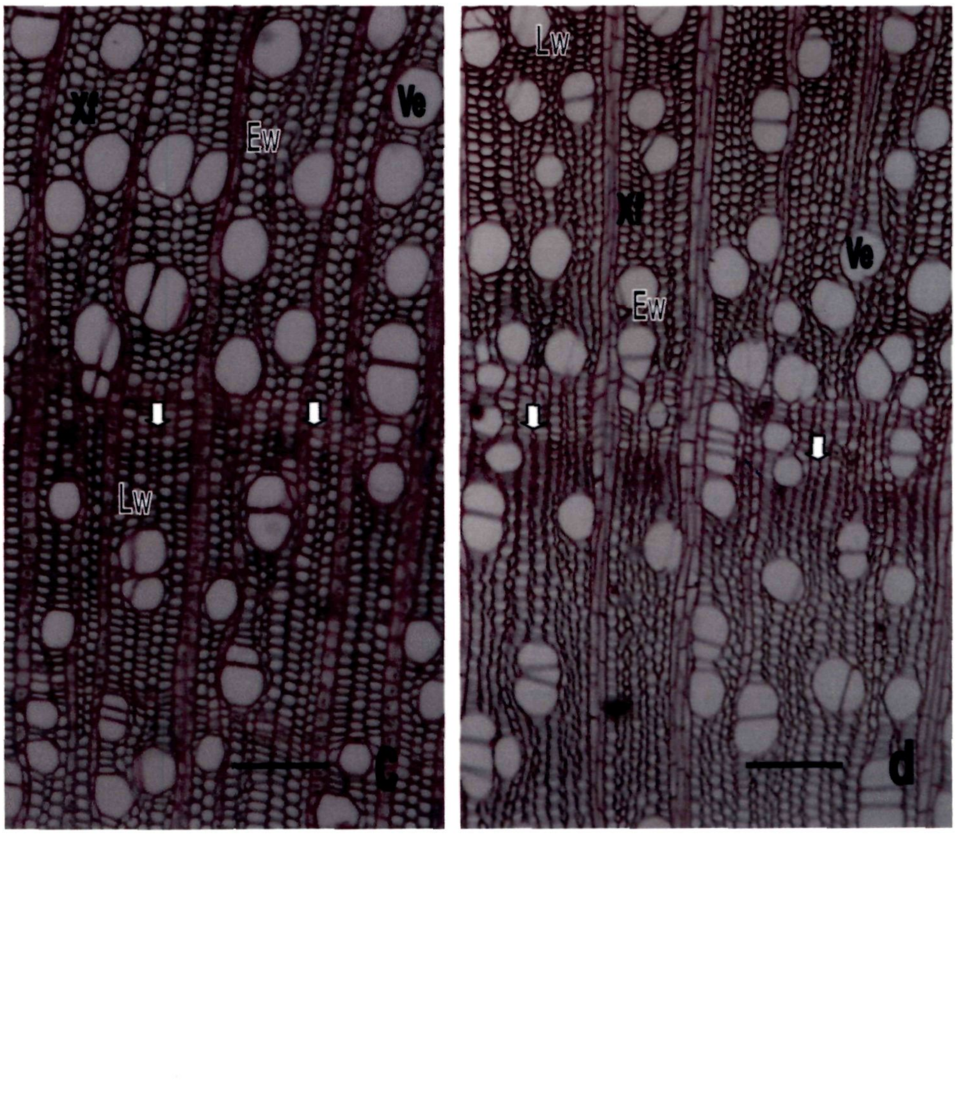
M. champaca Linn. and ***M. oblonga*** Wall. Ex Hk. f.

Macerated vessel elements of *M. oblonga*



Transverse section of wood

M. champaca *M. oblonga*



CHAPTER - 8

General Discussion

A survey of literature reveals that the organization of vascular cambium, its activity and duration have not been studied in detail on the primitive angiopermouse trees like *Dillenia indica, Exbucklandia populnea, Magnolia grandiflora, Michelia champaca,* and *Michelia oblonga* growing in sub-tropical moist forest of North-East India. Of course, Venugopal and Liangkuwang (2007) have published on seasonal cambial activity, duration of xylem production in relation to climatic factors in *D. indica*, a sub-tropical tree of North-East India. A few studies has been carried out in the sub-tropical trees, teak (Pumijumnong et al. 1995b), *Pinus merkusii* and *P. kesiya* (Pumijumnong and Wanyaphet 2006), growing in the South East Asia with particular emphasis on the dendrochronological aspects.

In India, the trees growing in sub-Himalayan regions and western region have been studied in the following plants: *Dalbergia sissoo* (Paliwal and Prasad 1970), *Hevea brasienlensis* (Rao 1972), *Polyalthia longifolia* (Ghouse and Hashmi 1979), *Acacia nilotica* var. *telia* and *Prosopis spicegera* (Iqbal 1979), *Gmelina arborea* (Dave and Rao 1982a), *Mangifera indica* (Dave and Rao 1982b), *Prosopis spicigera* (Iqbal and Ghouse 1982; Iqbal and Ghouse 1985b), *Streblus asper* (Ajmal and Iqbal 1987a), *Ficus rumphii* (Ajmal and Iqbal 1987b), *Tectona grandis* (Rao and Dave1981; Priya and Bhatt 1999; Rao and Rajput 1999; Rajput et al. 2005), in some species of *Altemanthera* and *Achyranthes aspera* (Rajput and Rao 2000a), *Acacia nilotica* (Rajput and Rao 2000b), *Tamarindus indica* (Rajput and Rao 2001), *Acacia nilotica* (Rao and Rajput 2001a), *Azadirachta indica* (Rao and Rajput 2001b), *Ailanthus excelsa* and *Artocarpus heterophylla* (Yadav et al. 2002), *Dalbergia sissoo* (Rajput et al. 2004).

In the peninsular India, both deciduous and evergreen trees have been studied in *Albizzia lebbeck*, *Tectona grandis*, *Dalbergia sissoo, terminalia crenulata*, *Calophyllum inophyllum*, *Mangifera indica* and *Morinda tinctora* (Venugopal 1986; Venugopal and Krishnamurthy 1987; Priya and Bhatt 1999).

However, there is lacunae in the cambial activity and wood formation in the Eastern part of Indian sub-continent. Therefore the present study has been carried out on the following primitive angiospermous plants: *Dillenia indica, Exbucklandia populnea, Magnolia grandiflora, Michelia champaca* and *Michelia oblonga* growing in sub-tropical wet forest of Meghalaya, North-East India.

The present investigation on five primitive angiospermous trees revealed that the significant relationship exist between the formation of new leaves and buds and cambial reactivation during the month of March (beginning of pre-monsoon or summer) in *E. populnea, M. champaca* and *M. oblonga;* April (middle of pre-monsoon or summer) in *M. grandiflora* and in the month of May (end of pre-monsoon or summer) in *D. indica.* The further continued activity of cambium was seen up to November in *E. populnea, M. champaca* and *M. grandiflora,* whereas the cambial activity extended up to the end of October and December in *M. oblonga* and *D. indica* respectively. In (pre-monsoon or summer), it is a crucial time for up-regulation of photosynthesis in the overwintered leaves and the leaves completely down-regulate photosynthesis during winter. The timing of vegetative bud break, different phenological events, initiation of cambial activity in these five plants are shown in Figures 4.1, 5.1, 6.1, 7.1A and 7.1B.

The cambial reactivation and xylem differentiation were seen in the month of May (end of pre-monsoon or summer), 15 days after the onset of bud breaks during the middle of April in *D. indica*. In *E. populnea, M. champaca* and *M. oblonga,* the reactivation of cambium as well as xylem differentiation were seen in the month of March (beginning of pre-monsoon or summer) in which the flushing of new leaves and buds was observed in the middle of February. In *M. grandiflora* also the cambial activity was started in the month of April (middle of pre-monsoon or summer) whereas the flushing of new leaves and buds was observed two weeks before the initiation of cambial activity i.e. in the middle of March. Therefore, in all these plants, the reactivation of cambium started generally in pre-monsoon or summer i.e. in between March to May. However, irrespective of all the plants, the flushing of leaves and buds was noticed two weeks before the onset of cambial reactivation.

A close association between the cambial activity and formation of new young leaves and buds was recorded in many forest tree of tropical, sub-tropical, arid and semi arid regions like, *Pyrus communis* and *Pyrus malus* (Evert 1961, 1963), *Pinus strobus* (Murmanis 1971), *Quercus boissieri*, *Pistacia alantica* (Fahn and Werker 1990), *Rhododendron arboretum* (Paliwal and Paliwal 1990), *Cedrela fissilis* (Marcati et al. 2006), *Polyalthia longifolia* (Ghouse and Hashmi 1979), *Gmelina arborea* (Dave and Rao 1982a), *Tectona grandis* (Rao and Dave 1981; Venugopal and Krishnamurthy 1987; Priya and Bhatt 1999; Rajput et al. 2005), *Acacia nilotica* (Rao and Rajput 2001a), *Azadirachta indica* (Rao and Rajput 2001b), *Dalbergia sissoo* (Paliwal and Prasad 1970; Venugopal and Krishnamurthy 1987; Rajput et al. 2004), *Aesculus hippocastanum* (Barnett 1992), *Larix leptolepis* (Oribe et al. 1993). In some species, such as *Prosopis spicigera* (Iqbal and Ghouse 1982) and *Streblus asper* (Ajmal and Iqbal 1987a), new leaf formation and cambial reactivation was separated by a gap of several weeks. Amobi (1974) found that the initiation of the cambial activity was correlated with bud break and leaf formation. Iqbal and Ghouse (1985b) have concluded that phenology of the plants and local climatic conditions affect the cambial activity. Bud burst and development of new leaves are related to cambial reactivation and xylem differentiation (Aloni 1991; Schrader 2003; Barlow and Powers 2005). Wareing et al. (1964) and Roberts (1976) reported that the young leaves and bud is the site of IAA and GA3 synthesis and their synergistic effect influence the cambial reactivation during the formation of leaves and buds. Reactivation of the cambium in different months in the same species growing under different local climatic condition was reported in some evergreen species (Zimmerman and Brown 1971; Rao and Rajput 1999, 2001a, b; Rajput and Rao 2000 b, 2001), but such comparative studies for the sub-tropical wet forest are lacking.

There was only one flush of activity of the vascular cambium in a growth period in *D. indica, E. populnea, M. grandiflora, M. champaca* and *M. oblonga*. The activity of vascular cambium has been observed for nine months in *E. populnea* from March to November (beginning of pre-monsoon or summer to end of retreating monsoon); eight months in *M. grandiflora* from April to November (middle of pre-monsoon or summer to end of retreating monsoon). These two plants viz. *E. populnea* and *M. grandiflora* are evergreen type with periodic growth. Here, the trees are truly evergreen. Major but not total leaf fall were started from February to April in *E. populnea* and from March to June in *M. grandiflora*. During this period, there was a concurrent flushing of new leaves and buds and it started from middle of February in *E. populnea* and from middle of March in *M. grandiflora* while the older leaves are still falling. Therefore, *E. populnea* and *M. grandiflora* belongs to evergreen leaf exchange type with periodic growth according to Longman and Jenik (1987). These species belong to type B based on their foliar behaviour. Fahn et al. (1968) and Dave and Rao (1982b) found that in certain evergreen trees, the correlation between bud break and cambial reactivation was not always clear. Amobi (1974) and Ghouse and Hashmi (1979) observed a positive correlation between the two in certain Nigerian trees and *Polyalthia longifolia* respectively. However, Paliwal et al. (1975) failed to find a relationship between cambial reactivation and bud breaks in *Polyalthia longifolia*. In both evergreen trees studied at present, increased intensity of cambial activity was noticed, immediately after the major bud break.

The cambial activity continuous for eight months in *D. indica* from May to December (end of pre-monsoon to the beginning of winter season), and dormancy from January to April (middle of winter to the middle of pre-monsoon or summer season) is imposed strongly by climatic condition. The cambial activity was observed for nine months in *M. champaca* and eight months in *M. oblonga*. In both the trees, it was started in March (beginning of pre-monsoon or summer) and continued up to October (beginning of retreating monsoon) in *M. oblonga* and November (end of retreating monsoon) in *M. champaca.* The cambium remain dormant for four months from November to February (end of retreating monsoon to end of winter) in *M. oblonga* and for three months from December to February (winter) in *M. champaca.*

In *D. indica,* Leaf fall started from February and complete defoliation resulted in the tree being barren for a brief period of first 15 days during the month of April. Leaf fall was initiated from November in *M. oblonga* and from December in *M. champaca* and leaf fall continued up to end of January in both the plants. In both the plants, complete defoliation was observed and the trees were barren in the first two weeks in February. Therefore, *D. indica, M. champaca* and *M. oblonga* growing in the sub-tropical climate of northeast India belongs to brevi-deciduous type (William et al. 1997; Borchert 1999). It simulates the phenology of tropical deciduous tree (Philipson et al. 1971; Dave and Rao 1982a; Fahn 1982; Liphschitz and Lev-Yadun 1986; Venugopal and Krishnamurthy 1987; Rao and Rajput 2001a, b).

The relation between the flowering /fruiting and cambial activity had not been adequately studied in the past. Reinders-Gouwentak (1965) and Amobi (1974) recorded that inflorescence induced wood formation in *Hidelgardia barteri* during leaf flushes (see also Philipson et al. 1971). Amobi (1974) found that in certain Nigerian mangrove trees irregular flushes of flower buds resulted on corresponding number of growth rings. In all the material studied at present, flowering coincided with the period of enhanced cambial activity. Even in the brevi-deciduous trees it could not be categorically proved that cambial reactivation and enhanced activity were due to flowering since the latter overlapped with vegetative bud break. Until many others trees are studied, the effect of flowering on cambial reactivation, if any, should be accepted only tentatively.

The cessation of cambial activity and its relation to abscission of older leaves was observed in the brevi-deciduous species i.e. *D. indica, M. champaca* and *M. oblonga*. Fruiting and heavy seeding have been reported to reduce the level of cambial activity markedly (Glock 1955). This was ascribed to competition for carbohydrates by the developing fruits and seeds on the one hand and the cambial tissue on the other. There was, however, no correspondence between fruiting and cambial deactivation neither in the brevi-deciduous nor in the evergreen trees studied at present.

Although a mild correspondence was noticed between periods of leaf fall and cambial dormancy in the evergreen plants studied at present only during the months of February and March in *E. populnea* and *M. grandiflora* respectively. The past literature is very silent on this issue. In the Oak tree (Longman and Coutts 1974), cambial division stopped long before leaf senescence. Since abscissic acid produced by senescing mature leaves was known to be important in promoting leaf abscission and since it was strongly implicated in the cessation of cambial activity (Roberts 1976), it is probable that in plants studied at present leaf fall period was found to be same as the period of cambial deactivation. Much more intensive studies are needed to verify the role of abscissic acid in cambial deactivation.

In all the investigated plants viz. *D. indica, E. populnea, M. grandiflora, M. champaca* and *M. oblonga,* the vascular cambium was non-storied type. The vascular cambium was comprised of fusiform and ray initials in all the plants. In *E. populnea,* the ray was mostly uniseriate, while in *D. indica,* the rays were composed of uniseriate and multiseriate types. In *M. grandiflora, M. champaca* and *M. oblonga,* uniseriate, biseriate and multiseriate rays were noticed. Attention has already drawn by the previous workers to the difference in thickness of radial and tangential walls of fusiform initials (see Catesson 1980). The tangential wall was always thinner than the radial wall irrespective of the season. Cytochemical, physical and structure difference were also shown to be present between these two walls (Catesson and Roland 1981; Benayoun et al. 1981). Large portion of radials walls were devoid of cellulose and fibrillar hemi-cellulose, which characterize the tangential walls (Benayoun et al. 1981). In all the materials studied at present, tangential wall is thinner than the radial walls.

The most characteristic feature of the radial walls of the fusiform initials was their beaded nature. This fact was observed by Bailey (1930). Bailey attributed that, the beading was due to the unequal thickening of portion of radials walls. Barnett and his school, however doubted the presence of beads in true cambial initials and stated that the beads in facts belong to the enlarging derivatives (Barnett 1981). In all the five plants studied here at no time fusiform initials were free of beads. Therefore, it is contended here that beads walls, characterize true cambial initials contrary to the view point of Barnett and his school. A careful review of previous literature and illustration provided therein revealed that true fusiform initials were in fact characterized by their beaded walls (Paliwal and Prasad 1970; Ghouse and Yunus 1974a, b; Goosen and Van Spronsen 1978; Timell 1980; Venugopal and Krishnamurthy 1987).

Schacht (1856) was perhaps the first to report multinucleate condition in the fusiform initials. This was followed by the similar report of Russow (1882) in some pines. Strasburger (1891) and Bailey (1920c) questioned this and stated that these two reports were due to erroneous observation. Subsequently, in *Solanum melongena* (Patel 1975), *Psidium guava* (Ghouse and Khan 1977), *Polyalthia longifolia* (Ghouse and Hashmi 1979), *Tectona grandis* (Dave and Rao 1981), *Acacia nilotica* var. *telia* (Iqbal and Ghouse 1987) and in several plants like *Albizzia lebbeck, Dalbergia sissoo, Tectona grandis, Terminalia crenulata, Mangifera indica* and *Morinda tinctoria* (Venugopal and Krishnamurthy 1987), multinucleate condition of fusiform initials was reported. The present study has confirmed the multinucleate condition of fusiform initials.

Several authors have reported the following numbers: 2 to in 5 in *Solanum melongena* (Patel 1975), 1 to 5 in *Psidium guava,* (Ghouse and Khan 1977), up to 5 in *Polyalthia longifolia* (Ghouse and Hasmi 1979), 1 to 5 in *Tectona grandis* (Dave and Rao 1981), 1 to 5 in *Acacia nilotica* var. *telia* (Iqbal and Ghouse 1987) and in several plants like *Albizzia lebbeck, Dalbergia sissoo, Tectona grandis, Terminalia crenulata, Mangifera indica* and *Morinda tinctoria* (Venugopal and Krishnamurthy 1987). In all the materials studied at present viz *D. indica, E. populnea, M. grandiflora, M.*

champaca and *M. oblonga,* 1 to 3 nuclei were of common occurrence, more than this numbers was infrequent. The least number of nuclei or sometimes a single nucleus was observed in the dormant fusiform initials. The nuclei vary greatly in their shape. Spherical to thread like nuclei with all intergrades were recorded, as in our materials, in all the materials of the above investigators. It should be mentioned here that in spite of the fusiform initials being multinucleate the derivative elements both on the xylem and phloem side generally had a single nucleus. However, Venugopal and Krishnamurthy (1984) reported multinucleate condition in the differentiating secondary xylem elements of *Dalbergia sissoo*. It is difficult to explain the purpose of multinucleate condition of the fusiform initials. Probably it has no significant in view of the fact that the immediate derivatives formed by both additive and multiplicative divisions of the fusiform initials always have a single nucleus. Much more intensive studies are necessary to understand this phenomenon.

In earlier literature one or more of the following changes have been recorded during reactivation after dormancy: 1. Increase in the number of cell layers in the cambial zone. 2. Swelling of cambial cells. 3. Increased in width of cambial zone consequent on (1) and (2). 4. Increased vacoulation of the cytoplasm of fusiform initials. 5. Clearing of cytoplasm and decrease in storage substance. 6. Elongation of fusiform initials. 7. Thinning of radial walls. 8. Wider separation of beads on the radial walls of fusiform initials and 9. Onset of periclinal division leading to the formation of secondary xylem and phloem. All the above changes were noticed during cambial reactivation in March in *E. populnea, M. champaca* and *M. oblonga*, April in *M. grandiflora* and May in *D. indica*.

There is a general agreement that swelling of fusiform initials represents the first sign of reactivation of the vascular cambium (Philipson et al. 1971; Catesson 1974; Ghouse and Hashmi 1979; Dave and Rao 1982a, b; Ajmal and Iqbal 1987b; Paliwal and Paliwal 1990; Priya and Bhat 1999). In all the plant studied at present swelling was found to be the first step in cambial reactivation and accounted for the increase in width of cambial zone. A few people have failed to notice swelling of cambial cells prior to reactivation (Evert 1963; Derr and Evert 1967; Davis and Evert 1968). Even in case where swelling was not apparent, the individual cambial cells underwent expansion before dividing periclinally (Savidge and Wareing 1981). There appears to be no basic difference between the two categories mentioned above as swelling varied only temporarily. Swelling therefore appears to be a necessary prerequisite for the cambial cells to undergo periclinal divisions. Many investigators have considered the numbers of layers in cambial zone as the most reliable criterion to distinguish cambial dormancy, reactivation and peak activity (Rao and Dave 1981; Rao and Rajput 2001b), a fact also supported by the present study. The cambial zone contained fewer layers during dormancy than during active period. Actual figures have been given for dormant and active cambia of numbers of dicots and gymnosperms (Timell 1980). The dormant cambium of the material investigated here invariably contained 3-4 layers in all the plants, while at the peak activity, up to 7-8 layers in *M. grandiflora*, up to 8-9 layers in *D. indica, E. populnea, M. champaca* and *M. oblonga* were noticed. The maximum number of layers recorded elsewhere was 24 in *Tectona grandis* (Rao and Dave 1981).

The cytological details of perilcinal and anticlinal division of all the plants investigated were similar to those already described for other plants (1920a, c; Goosen de-Roo and Van Spronsen 1978). Most frequent periclinal division was reported to be nearer to the xylem side of the cambial zone by Newman (1956) and Bannan 1950, 1955). Although the present study indicated greater frequency of cell division away from the centre of the cambial zone (the initials), it did not also lend support to the contention that division were always most frequent on the xylem side. In fact the frequency of periclinal division depended on the type of vascular tissue produced. But periclinal divisions were never seen to the maximum at the centre of the cambial zone where the "initials" divided periclinally very less and were comparatively quiescent.

The location of initial periclinal divisions subsequent to dormancy was also a matter of dispute, some claiming it near the xylem (Bannan, 1955, 1962; Savidge and Wareing 1981). Some near the phloem (Tucker and Evert 1969) and others reporting is to be evenly distributed across the cambial zone (Evert 1963; Derr and Evert 1967). The occurrence of pseudotransverse type of anticlinal division has been noticed after the peak period of cambial activity i.e. during the declining phase of cambial activity in all the plants. The length and pitch of the sigmoidal partition wall after pseudotransverse division vary from short and almost transverse divisions transverse to highly inclined (Philipson et al. 1971). A similar range was observed in the different plant studied, some showing almost transverse wall and other inclined wall. The direction of the orientation of the spindle and inclination of the new cell plates were described to be similar in adjacently placed anticlinally dividing fusiform initials often resulting in spiral grains (Hejnowicz 1961). Exceptions to this were reported to be present (see Philipson et al. 1971). In *D. indica and M. champaca*, studied at present adjacent fusiform initials often differed considerably with reference to the orientation of nuclear spindle and the direction of inclination of partition walls.

Anticlinal divisions were reported to be usually restricted to the end of the end of the growth season (Davis and Evert 1970; Kozlowski 1971; Venugopal and Krishnamurthy 1992) and this was often given as an evidence for the presence of shorter fusiform initials during the approach of dormancy. While it is true for all plants studied that anticlinal divisions were predominantly seen at the same time of least cambial activity, these divisions nevertheless were not restricted to this period.

Our results reveal the occurrence of annual fluctuations in the lengths of fusiform cambial cells in *D. indica, E. populnea, M. grandiflora, M. champaca* and *M. oblonga*. The length of fusiform intials was more during active period than during dormancy. It has been suggested that the annual fluctuations in the mean length of fusiform cambial cells might be due to changes in the frequency of anticlinal divisions (Swamy et al. 1960; Bannan 1967, 1970; Ajmal and Iqbal 1987b). Recently Kojs et al (2004b) reported that the readjustment of cambial intials in *Wisteria floribunda* for development of storied structure. Jura et al. (2006) also reported apical intrusive growth of fusiform initials along the tangential walls of adjacent fusiform initials.

Starch is the common major storage product in all the five plants which is mainly observed towards the xylem and phloem sides adjacent of cambial zone. Phenolic contents are also present in *D. indica* and *E. populnea.* Apart from polyphenols, cystolith in *E. populnea* and raphides in *D. indica* have also been observed in the phloem, mainly in the phloem rays during the dormant period. But in *M. grandiflora, M. champaca* and *M. oblonga,* the ergastic substances are absent even during the dormant period. The same feature was also recorded in *Salix viminalis* (Sennerby-Forsse 1986). Robards and Kidwai (1969) observed oil droplets and protein bodies by histochemical methods as well as ultrastructural level in resting cambium of *Salix fragilis.*

The storage product such as starch and crystal of calcium oxalate stored in the cambial zone and its derivatives during the dormant period of the current year, probably served as the source material for the new cell wall synthesis for the next year growth (Essiamah and Eschrich 1985; Sauter 1966; Parker 1960; Pomeroy and Sminovitah 1971; Tsuda and Shimaji 1971; Priya and Bhat 1999; Rajput and Rao 2000). The maximal and minimal starch content in the xylem of *Abies balsamea* (L.) Mill. (Abietaceae) associated respectively with the period of cambial dormancy and reactivation (see also Riding and Little 1984; Parker 1960; Sauter 1966; Pomeroy and Siminovitch 1971; Tsuda and Shimaji 1971; Essiamah and Eschrich 1985). When there are two flushes of cambial activity and dormancy, the accumulation and depletion of starch and calcium took place twice a year (Venugopal and Krishnamurthy 1987). Marcati and Angyalossy (2005) reported the seasonal presence of acicular crystals in the cambial zone of *Citharexylum myrianthum*. Druses and acicular crystals have been found in the cambial zone in some species of Verbenaceae (Rao and Dave 1984; Deshpande and Vishwakarma 1992) and in other families (Rao and Menon 1989). Deshpande and Vishwakarma (1992) reported a correlation between the presence of crystals and cambium activity in *Gmelina arborea* (Verbenaceae).

Production of xylem has been recorded once in a year in *D. indica* from May to December end (end of pre-monsoon or summer to the beginning of winter), from middle of March to November end (beginning of pre-monsoon or summer to end of retreating monsoon) in *E. populnea* and *M. champaca*. In *M. oblonga,* xylem development was observed from middle of March to October end (beginning of pre-monsoon or summer to beginning of retreating monsoon), while in *M. grandiflora,* it was started from the middle of April to November end (middle of pre-monsoon or summer to end of retreating monsoon). A similar pattern of xylem production is seen in most of the plants (Philipson et al. 1971; Fritts 1976; Ajmal and Iqbal 1987b; Larson 1994; Iqbal 1994; Schweingruber 1996; Worbes 1995, 2002; Rajput and Rao 2001; Rajput and Rao 2001a, b; Rajput et al. 2004, 2005; Marcati et al. 2006; Pumijumnong and Wanyaphet 2006; Heinrichs et al. 2007). However, Liphschitz et al. (1981) have reported two flushes of xylem production in *Cupressus sempervirens* growing in mediterranean climate. Fahn (1958) has also reported two installment of xylem production in *Tamarix aphylla*, *T. jordanis* var.

negevensis, *T. gallica* var. *maris-mortui*. Multiple ring formation in correspondence with the number of bud break within a growth period was reported in many tropical plants (Amobi 1974; Venugopal and Krishnamurthy 1987). Rao and Rajput (2001a) observed single growth ring formation in *Acacia nilotica* (L.) Del. growing in dry deciduous forest even though this tree produced new leaves twice a year.

Though *D. indica* and *E. populnea* growing in the sub-tropical wet climate showed distinct annual rhythm in the cambial activity, it result in the formation of invisible growth ring annually. In *Quercus costaricensis*, growing in mountain sites in Costa Rica, no clear ring was formed even though it showed annual periodicity (Worbes and Junk 1989). In other three plants viz. *M. grandiflora, M. champaca* and *M. oblonga,* cambial activity showed distinct annual rhythm and resulted in formation of distinct growth ring. Annual tree ring formation relies on a shift of the cambium into dormancy once a year (Strasburger et al. 2002). The following examples support our observation on the formation of distinct growth rings in the sub -tropical wet climatic arborescent plants; *Tabebuia umbellate* (Callado et al. 2004), *Dalbergia sissoo* (Rajput et al. 2004), *Pinus sylvestris* and *Betula spp* (Schmitt et al. 2004), *Tectona grandis* (Rajput et al. 2005), *Pinus merkusii* and *Pinus kesiya* (Pumijumnong and Wanyaphet 2006), *Toona sinensis* and *Toona ciliate* (Heinrich and Banks 2006), *Cedrela fissilis* (Marcati et al. 2006), *Betelu papyrifera, Picea glauca, P. mariana, Pinus banksiana, P. resinosa,* and *Thuja occidentalis* (Heinrich et al. 2007).

Of the five trees species belonging to three families viz. Dilleniaceae, Hamamelidaceae and Magnoliaceae studied in the present investigation, the wood is diffuse porous type. Growth periodicity was observed in all the plants studied. Various anatomical features such as the presences of terminal parenchyma, initial parenchyma, radially compressed fibres and a lower frequency of vessel in the latewood are used to demarcate the growth ring in tropical wood (Chowdhury 1964; Carlquist 1980). In tropical trees, there are conflicting reports on growth periodicity with regard to absence or presence of growth rings (de Alvim 1964). Chowdhury (1961) recorded 25% of forest trees in India showed distinct growth ring, where diffuse porous species accounted for over 90% of all woody dicotyledons (Chowdhury 1939). In *D. indica* and *E. populnea* tangentially compressed of two to three layers of fibres and by narrow layers of radially compressed xylem fibres demarcate the growth ring respectively (Pearson and Brown 1981). In *M. champaca* and *M. oblonga* the growth ring were demarcated by initials parenchyma whereas it is demarcated by terminal parenchyma in *M. grandiflora*.

Shorter element characterized the end of xylem production, while the longer element marks the peak period of the cambial activity and xylem production. Simultaneously, the diameter of wood element was greater during the period when their production was at its peak and minimum during the period when the activity of cambium was the lowest. An analysis of earlier literature reveals that this trend is shown by the largest number of plants studied (See also Taylor 1976; Venugopal and Krishnamurthy 1987; Siddiqi 1991; Venugopal and Liangkuwang 2007), even though the presence of longer late wood fibres (Bisset and Dadswell 1950; Panshin and De Zeeuw 1980; Lopez-Ayala et al. 2006) and longer late wood vessel elements (Kitin et al 1999; Lopez-Ayala et al. 2006) has been recorded.

In the present investigated species of *D. indica, E. populnea, M. grandiflora, M. champaca and M. oblonga*, it was observed that the vessel elements were shorter than fusiform initials. This might be due to successive oblique or transverse division in the xylem mother cells prior to their differentiation into mature xylem elements as pointed out by Philipson et al. (1971). Ajmal et al. (1985) reported that the vessel element was shorter and broader than the fusiform initials in *Eucalyptus* sp. Various hypotheses have been proposed to explain differences in length between vessel elements and fusiform cambial cells. Some authors have attributed the shortening of vessel elements to a change in the position of the end wall during differentiation, while others have suggested that the shorter vessel elements might be due to shortening of the cambial initials through anticlinal divisions (for references, see Larson, 1994). Xylem fibres, in the present study exhibit increase in length over the fusiform intials. The gain in length is obviously due to apical intrusive growth experienced during differentiation. This view is supported by their having long and narrow tips with diverse shapes (Bailey 1920a; Anand et al 1979; Iqbal and Ghouse 1983).

Only few results have been published on the size relationship between cambial cells and their derivatives elements (Philipson et al 1971). Anand et al (1978) and Sharma et al. (1979) studied the size variations of cambial intials and their derivatives in xylem and phloem of *Dalbergia sissoo* and *Polyalthia longifolia* respectively. In the present investigation, the periodic changes in fusiform initials length appear to be reflected in the variation of xylem fibres and vessel elements. The reflection of length variation between xylem fibres and fusiform initials has also been recorded in Teak (Rao and Dave 1981). Bailey (1920a), who studied 13 species of gymnosperm and 54 dicotyledonous species from 14 families, provided extensive data on lengths of fusiform cambial cells. Bailey (1920 a) concluded that in most of the conifers, the tracheids are closely similar in length to, or slightly longer than fusiform initials from which they are derived. In dicotyledonous trees fibres were longer, while the vessel members were approximately of the same length as the fusiform initials.

The timing of reactivation, peak activity of cambium and xylem production and mean cell length in *D. indica, E. populnea, M. grandiflora, M. champaca* and *M. oblonga* were studied in relation to the various climatic factors such as monthly temperature (mean temperature, mean minimum temperature and mean maximum temperature), precipitation and relative humidity. Periodicity of cambial activity and xylem production is controlled by various environmental and physiological factors (Reinders-Gouwentek 1965; Philipson et al. 1971; Kramer and Kozlowski 1979; Venugopal 1986; Ajmal and Iqbal 1987a, b; Venugopal and Krishnamurthy 1987; Iqbal 1994; Larson 1994; Rao and Rajput 1999; Grotta et al. 2005; Gricar et al. 2006; Li et al. 2006; Yanez-Espinosa et al. 2006; Druart et al. 2007; Fonti et al. 2007; Begum et al. 2007). The monthly mean values of all the climatic factors are mentioned in Chapter 3 for two consecutive years 2002 and 2003 (See Table 3.1; Fig. 3.1).

The optimum temperature for the initiation of cambial activity and xylem differentiation is 18-21°C. In *D. indica*, the actual temperature 17.63°C (estimated temperature18°C- as mean minimum temperature) has been observed during the month of May, whereas in *E. populnea, M. champaca* and *M. oblonga,* the actual temperature is 18.43°C (as mean temperature) and fall during the months of March. While in *M. grandiflora,* it was observed in the month of April and the actual temperature is 20.91°C (as mean temperature). Therefore, it has been proved categorically that the optimum temperature for the initiation of cambial activity and xylem differentiation is 18-21°C, which is mean minimum temperature in *D. indica,* mean temperature in *E. populnea, M. grandiflora, M. champaca* and *M. oblonga*. These observations also proved that the interrelationships between bud break, initiation of cambial activity and xylem differentiation with monthly ambient temperature occurred during the pre-monsoon or summer season. The variation within these five plants regarding the timing of cambial reactivation in different months may be due to intrinsic factors of that particular plant.

There was a positive correlation between the cambial dormancy and absence of xylem production with the low temperature range during January to April in *D. indica*; December to February in *E. populnea* and *M. champaca*; November to February in *M. oblonga* and from December to March in *M. grandiflora*. Therefore, a rise in temperature is enough to induce the formation of new buds and foliage and thereby reactivate the cambium in all the investigated plants after dormancy. Higher temperature was reported to be conducive for cambial reactivation and xylem production in *Picea glauca* (Gregory and Wilson 1968). A similar view was expressed by (Kramer and Kolzowski 1979), that, temperature was a significant factor for bud break following reactivation and subsequent shoot growth. On the other hand, a rise and fall in temperature was reported to have no effect on the cambial activity in *Eucalyptus camaldulensis* (Waisel et al. 1966) and in *Cupressus sempervirens* (Lipschitz et al. 1981). High temperature is necessary for leaf initiation, favouring simultaneously cambial activity in *Tectona grandis* (Rao and Rajput 1999); *Aphananthe monoica, Pleuranthodendron lindenii* and *Psychotria costivenia* (Yanez-Espinosa et al. 2006). Temperature is also known to play an important role in activating the cambium in several temperate and tropical trees (*Streblus asper* (Ajmal and Iqbal 1987a), *Ficus rumphii* (Ajmal and Iqbal 1987b), *Cryptomeria japonica* (Oribe and Kubo 1997), *Azadirachta indica* (Rao and Rajput 2001b), *Betula papyrifera, Abies balsamea, Thuja occidentalis, Picea glauca, Picea mariana, Pinus banksiana* and *Pinus resinosa* (Tardif et al. 2001a), *Larix kaempferi* (Funada et al. 2002), *Quercus robur* (Horacek et al. 2003), (*Populus sieboldii* X *P. grandidentata*) (Begum et al. 2007), *Betelu papyrifera, Picea glauca, P. mariana, Pinus banksiana, P. resinosa,* and *Thuja occidentalis* (Heinrichs et al. 2007). It appears that temperature factor does not act independently and the law of limiting factor may be in operation (Coile 1936; Keen 1937). The mechanism by which higher temperature promoted the cambial reactivation in many tree is not clear.

On the basis of in vitro experiment, the increase in temperature was responsible for the release of auxin reserve from the tissue adjacent to cambium, which, in turn, activated cambium (Wort 1962). On the other hand, temperature had implicated in promoting vacuolation of fusiform initial and may likely to be the effect of increased temperature (Catesson 1962). It has already been demonstrated that the application of localized heating of stems induces localized cambial reactivation (Savidge and Wareing 1981; Barnett and Miller 1994; Oribe and Kubo 1997; Oribe et al. 2001, 2004; Begum et al. 2005; Griciar et al. 2006; Begum et al. 2007).

The role of rainfall on cambial behaviour and xylem production under drought condition was studied much more intensively than other factor in the past. Higher rainfall was reported to be conducive to cambial reactivation in several plants especially growing in the tropic and semi-arid climates (Glock1955; Reinders-Gouwentak 1965; Rogers 1981; Dave and Rao 1982a, b). The present study also shows that the mean rainfall has less correlation than mean minimum temperature in *D. indica* and mean temperature in *E. populnea, M. grandiflora, M. champaca* and *M. oblonga*. Rainfall probably is an important factor only in the regions where soil moisture content is dependent on rainfall (Rao and Rajput 2001a, b). This study has indicated that cambial reactivation, peak activity and xylem production were not limited by rainfall because these five plants grow only in limited habitat of sub-tropical wet forest of northeast India, with enough moisture throughout the year. In other words, these plants are not subjected to physical drought or water stress (Fahn 1959a, b; Amobi 1974). However, the monthly mean precipitation is below 50 mm during December to February (winter season) and the soil type is oxisols where moisture content is 30-40% (Pandey 2004; Porwal et al. 2000; Tripathi 2002; Brady and Well 2002). Moreover, this study site is located 60 km from

Cherrapunji, which is the region of highest rainfall (965 cm per year) in the world (Lal 2000; Anonymous 1971, 1972). It was observed that a higher mean minimum temperature in *D. indica* and mean temperature in *E. populnea, M. grandiflora, M. champaca* and *M. oblonga* generally favoured both cambial reactivation and xylem production. The other factors such as relative humidity and precipitation have little effect on the cambial periodicity and xylem differentiation in the trees growing in the sub-tropical wet climate.

CHAPTER - 9

Summary and Conclusion

The ability to incorporate external stimuli is reflected in different characteristics. The characteristic feature of growth ring is affected by the activity of the vascular cambium and this activity is controlled by many factors, both endogenous and exogenous. Climatic factors play a significant role in the activity of cambium. Therefore, in this thesis, five primitive angiospermous trees VIz. *Dillenia indica* Linn., *Exbucklandia populnea* (R. Br. Ex Griff.) R. W. Br., *Magnolia grandiflora* Linn., *Michelia champaca* Linn. and *Michelia oblonga* Wall. Ex Hk. f. were studied with reference to the following aspects:

1. To find out the cambial activity in relation to phenology and climatic factors
2. To study the seasonal variation in the structure, cytology and ergastic substance of the vascular cambium
3. Duration of wood production
4. Dimensional changes in the vascular cambium and its derivatives during different seasons

The interrelationship between different phenological events such as emergence of new leaves and buds, flowering, fruiting, seed dispersal and leaf fall, with environmental factors such as temperature, rainfall, relative humidity and periodicity of cambial activity was examined for all the five plants. It was observed that there was a clear cut correspondence between timing of vegetative bud break and cambial reactivation in all the five plants. Since the timing of flowering overlapped with the formation of new leaves, it could not be categorically proved that reproductive phase was really responsible for enhance activity of the cambium in these five primitive angiospermous trees.

It was observed that both cambial reactivation and peak activity were generally favoured by a higher mean temperature in all the investigated plants; a positive correlation also existed between the least cambial activity/dormancy and lower mean temperature. Relative humidity had no effect on cambial periodicity. The selected plants did not have any water stress because they are growing in the sub-tropical wet forests of North east India, where water was not at all a limiting factor. Moreover, this study site is located 60 km from Cherrapunji, which is the region of highest rainfall.

The vascular cambium of all the plants is non-storied type. The length of fusiform initials was highest in *Exbucklandia populnea* and lowest in *Michelia champaca*. The radial and the tangential wall of fusiform initials were primary in nature; however, the radial wall was not only thicker than the tangential wall but also always beaded. The beading was more prominent and closer to one another during dormancy than during active period of the vascular cambium. The fusiform initials of all plants showed multinucleate condition 2-3 in *D. indica, E. populnea* and *M. grandiflora,* 3-4 in *M. champaca* and *M. oblonga,* of varied shapes and sizes. Cambial reactivation was marked by radial swelling of cambial cells, resulting in increase of width of cambial zone. This was followed by periclinal division and as a result the numbers of layers in the cambial zone increase. There was also an increase in length of fusiform initials which consequently brought about the wide separation of the beads in the radial walls. The frequency of periclinal divisions was more during the active state of the vascular cambium, while towards dormancy the cambial zone exhibited predominantly anticlinal divisions in the plants.

In all these primitive angiospermous trees, the secondary xylem were diffuse porous wood. The end walls of the vessel elements in all the plants had a scalariform perforation plate. Growth ring was distinct and demarcated by initial parenchyma in *M. champaca* and *M. oblonga,* by terminal parenchyma in *M. grandiflora.* But the growth ring is indistinct, occasionally indicated by layer of radially compressed fibres in *E. populnea,* delimited by smaller vessel and denser fibrous tissue in *D. indica.* Fibres tracheid and nucleated xylem fibres was present in *E. populnea.*

The duration of xylem production was about eight and half months in *E. populnea* and *M. champaca*, about eight months in *D. indica,* about seven and half months in *M. grandiflora* and *M. oblonga.* The length of fusiform initials and their derivatives like xylem fibres and vessel elements was maximum during the peak activity of the vascular cambium and was minimum during cambial dormancy. The trend in variation in the length of different vascular elements followed closely the trend in variation in length of fusiform initials from which they were derived.

Conspicuous changes could be found on the content of secondary metabolites (ergastic substances) during the approach of dormancy as well as during cambial reactivation and peak cambial activity. In *D. indica,* starch grain, polyphenol and tannin content are the major reserved product.

Phenolic contents were present in both active and dormant cambium. In *E. populnea*, the amount of starch grain, phenolic contents and crystal of calcium oxalate (cystolith) appeared in large quantity with the approach of dormancy but disappear slowly with the onset of cambial reactivation and disappear in the active period. The starch grains were the major food reserved in *M. grandiflora*, *M. champaca* and *M. oblonga*. Probably, these substances form the source of new wall materials of the new cambial derivatives.

Conclusion

- It was observed that the phenological events especially sprouting of new leaves and buds had a close relationship with cambial reactivations.
- The young leaves and buds are the sites of growth hormones which regulate the reactivation of cambium and xylem differentiation.
- During the peak activity, the foliage leaves up regulate the photosynthesis which provides necessary materials for cell wall synthesis and differentiation. On the contrary during dormancy the senescence of leaves coupled with leaf fall irrespective of brevi-deciduous and evergreen trees, down regulate the photosynthetic machinery.
- Conspicuous changes could found in the content of storage and ergastic materials of vascular cambium and xylem tissues towards the approach of dormancy.

- The reduction in the size of vascular cambial cells and xylem elements with the approach of dormancy may be gradual or abrupt depending upon the plant.
- The fusiform initials were multinucleate in all the investigated plants.
- In the dormant cambium, the cell walls are relatively thicker than those cell walls in the active cambium, although in both the radial walls are thicker than the tangential ones. In active phase of growth, with cell hydration and swelling, the walls become thinner, the primary pit-field are drawn further apart and the nuclei lose their chromaticity. In the dormant cambium, the cells contain many small vacuoles, large amount of tannins, protein bodies and /or lipid droplets.
- The rate of cell production is faster than the rate of differentiation of new derivatives in the active cambium, which result in increase of cambial zone. When the rate of division decrease and differentiation proceeds faster than division. The cambial zone becomes narrow.
- The periodicity of cambial activity and the formation of growth rings in the xylem are most pronounced in *M. champaca*, *M. oblonga* and *M. grandiflora*, but in *D. indica* and *E. populnea*, the growth rings are not visible with naked eye. The growth periodicity simulates to those trees growing in temperate region.
- Xylem production was noticed about eight and half months in *E. populnea* and *M. champaca*, about eight months in *D. indica*, about seven and half months in *M. grandiflora* and *M. oblonga*.

- The length of fusiform initials and its derivatives followed the same trend with respect to the external environmental factors and to some extent leaf phenology rather than reproductive phases.
- It was also observed that both the cambial reactivation after dormancy and peak cambial activity were generally favoured by ambient air temperature of 18-21° C. Further activity of vascular cambium and differentiation of xylem occurred due to the synergistic effect of both temperature and precipitation. Relative humidity had little/no effect on cambial activity and xylem formation. Cambial dormancy was imposed by the fall in mean temperature.
- Multiple (partial) regression analysis have shown that monthly mean minimum temperature in *D. indica* and mean temperature in *E. populnea*, *M. champaca*, *M. oblonga* and *M. grandiflora* is an important factor for cambial reactivation and xylem production. The optimum temperature being in between 18-21° C.

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B.Sc	1999	NEHU	Bot, Che, Zoo	I st& 63.12
M.Sc	2001	NEHU	Botany	I st & 68.11
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Cambial activity and annual rhythm of xylem production of elephant apple tree (*Dillenia indica* Linn.) in relation to phenology and climatic factor growing in sub-tropical wet forest of northeast India

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Abstract The interrelationship between phenological events, climatic factors, periodicity of cambial activity and seasonal production of xylem was examined in *Dillenia indica* L. (Dilleniaceae) growing in sub-tropical wet forest of Meghalaya state, India. The reactivation of cambial activity was seen in the first week of May, 15 days after sprouting of new leaves and buds. The activity of cambium and xylem production gradually declined toward December and ceased from January to April end. There was correlation between leaf fall and cambial dormancy. It was evident from the correlation and regression analysis, the relationship between cambial activity, xylem production with climatic factors, the monthly mean minimum temperature plays an important role for the cambial activity and xylem production rather than influence by rainfall and relative humidity in *D. indica* L. The data were discussed in the light of cambial activity, xylem production and phenological events.

Keywords *Dillenia indica* L. · Vascular cambium · Annual rhythm of xylem production · Sub-tropical wet forest · Multiple (partial) regression analysis · Northeast India

Introduction

The activity of vascular cambium is not uniform throughout the year and determined by the interaction of internal and external factors (Philipson et al. 1971; Larson 1994; Iqbal 1994; Grotta et al. 2005). The majority of past studies on cambial activity are pertained to plants growing in temperate region with definite seasonal climates (Bailey 1920; Bannan 1955, 1962; Antonova 1996, 1997; Antonova and Stasova 1997; Rensing and Samuel 2004). The seasonal variations of cambial activity and annual rhythm of xylem and phloem differentiation in tropical trees, semi-arid and arid regions have been studied in quite a number of plants (Coster 1927, 1928; Chowdury 1939, 1940, 1941; Koriba 1958; de Alvim 1964; Fahn et al. 1968; Amobi 1974; Ghouse and Hashmi 1978; Denne and Dodds 1981; Dave and Rao 1982; Venugopal 1986; Venugopal and Krishnamurthy 1987; Creber and Chaloner 1990; Larson 1994; Priya and Bhat 1999; Borchert 1999; Rao and Rajput 2001a, b). The effect of genetic and environmental factors on shoot growth and xylem formation has been studied in the West African tropical tree *Terminalia superba* Engl. and Diels (Combretaceae) (Longman et al. 1979). Periodicity of wood formation in twigs of 11 tropical trees was studied in different ecological areas, such as lowland rainforest, savannah and mangrove swamps in Nigeria (Amobi 1974). Recently, the wood production has been estimated in the natural forest stand in Cameroon by using tree ring analysis (Worbes et al. 2003). Cambial activity and annual rhythm of xylem development in trees and shrubs of desert plants *Tamarix aphylla* (L.) Karst., *T. jordanis* var. *negevensis*, *T. gallica* L. var. *maris-mortui* (Gutm.) Zoh., *T. jordanis* Boiss. var. *negevensis* Zoh., (Tamaricaceae) have been studied in Israel (Fahn 1958). In the Southeast Asian countries, a sizable number of trees have been studied. The earliest report on the growth of *Tectona grandis* L.f (Verbenaceae) was studied by Brandis (1856) by measuring ring width (Mariaux 1981). The correlation between cambial growth and rainfall has been assessed in the lowland dipterocarp forest of Peninsular Malaysia (Killmann and Thong 1995). Cambial activity, development of xylem

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and phenology have been studied in *Azadirachta indica* L. (Meliaceae) (Rao and Rajput 2001a, b), *Tectona grandis* L.f. (Berlage 1931, Rao and Dave 1981, Venugopal and Krishnamurthy 1987, Rao and Rajput 2001a, b, 1999, Priya and Bhatt 1999), *Acacia nilotica* (L.) Del., *Albizzia lebbeck* Benth. (Mimosaceae) (Rao and Rajput 2000, 2001a, b, Venugopal and Krishnamurthy 1987), *Dalbergia sissoo* Roxb. ex DC. (Fabaceae) (Ghouse and Yunus 1974), Venugopal and Krishnamurthy 1987) *Polyalthia longifolia* Sonn. (Annonaceae) (Ghouse and Hashmi 1978), *Mangifera indica* L. (Anacardiaceae) (Dave and Rao 1982, Venugopal and Krishnamurthy 1987), *Calophyllum inophyllum* L. (Clusiaceae), *Morinda tinctoria* Roxb. (Rubiaceae), *Terminalia crenulata* Roth. (Combretaceae) (Venugopal and Krishnamurthy 1987).

On the basis of phenological rhythms, cambial activity and climatic conditions, the tropical trees have been classified into four types (evergreen, stem succulent, deciduous and brevideciduous) growing under the same ecological conditions (Borchert 1999). The effect of flood on tree growth in the Amazon forest in relation to phenology and analysis of ring has been studied in detail (Schongart et al. 2002, Dezzeo et al. 2002, Worbes 1999). However, studies on the seasonal activity of vascular cambium and production of xylem in relation to different climatic factors in sub-tropical wet forest are scarce. Therefore, the present study on annual rhythm of cambial activity and differentiation of xylem in relation to phenology as well as the climatic improves our further understanding of tree growth in *D. indica* L. growing in sub-tropical wet forest of northeast India. Furthermore, this type of study is applicable to estimate tree's productivity and growth as well as evaluating past and present forest environments through tree ring research (Fritts 1976, Worbes 1995, 2002, Schweingruber 1988, 1996).

Material and method

Study area and climate

The upland area, where most of the sub-tropical mixed forest exists, was selected for the study in Botanical Survey of India, Experimental Garden, Shillong (25°34′N and 91°53′E) with an elevation of 1,100 m a.s.l. The elevation above sea level is characterized by mountain climate or wet hill forest climate with low temperature and relatively high precipitation (Lal 2000, Worbes and Junk 1989). As the altitude increases, various climatic elements such as pressure, temperature and precipitation undergo well-defined changes (Lal 2000). The soil is loamy, reddish brown in colour and lateritic in origin. The pH ranges from 5.9 to 6.2 (Singh 1996, Porwal et al. 2000, Mishra et al. 2003, 2004). Climatologically, this study area belongs to the sub-tropical wet climatic region (Champion and Seth 1968). On the basis of variation of temperature, rainfall and wind, the year in the region may be divided into four distinct seasons (1) winter (December–February), (2) pre-monsoon or summer (March–May), (3) monsoon (June–September) and (4) retreating monsoon (October and November) (Porwal et al. 2000, Dhirendra Singh 2002), Tripathi et al. 2004). This region receives abundant southwest monsoon from June to October. Highest rainfall is recorded in the months of June and July. The mean temperature ranges from 5 to 15°C in winter and from 20 to 25°C during summer. The data on the climatic factors were collected from Central Seismological and Meteorological observatory, Shillong station, Government of India. Monthly mean, mean maximum, mean minimum temperature, rainfall and relative humidity for the years 2002 and 2003 were chosen for this study (Fig. 1).

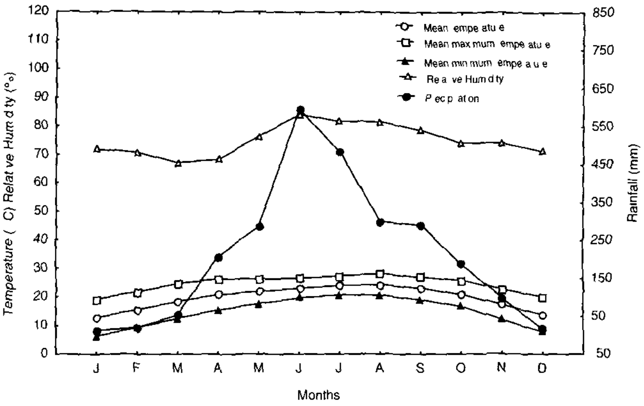


Fig. 1 Showing different climatic factors in the study area of *D. indica* during 2002–2003

Field observations

Periodic collection of twigs measuring from 1.5 to 2 cm in diameter was made from ten plants growing in the Botanical Survey of India, Experimental Garden, Shillong. They were fixed in FAA (formalin-aceto-alcohol), Glutaraldehyde and Cornoy's fluid in the field itself at every fortnight interval for two consecutive years 2002 and 2003. The timing of different phenophases such as leaf fall, leaf flush, flowering, fruiting and seed dispersal was recorded during this period (Fig. 8).

Analyses in laboratory

The materials were processed through customary method of dehydration, paraffin embedding and sectioning at a thickness of 8–10 μm. The sections were stained either with tannic acid and ferric chloride counterstained with lacmoid blue (Cheadle et al. 1953) or with toluidine blue O (Feder and O'Brien 1968). For micro measurement, recently formed xylem tissue was carefully teased out and macerated according to Jeffrey's method (Berlyn and Miksche 1976). Total starch content and phenol were localized by using

Table 1 Quantitative data of vascular cambium and their derivatives in *Dillenia indica*

Months	Numbers of cambial layers	Average cambial zone width (μm)	Average length of fusiform initial (μm)	Average width of differentiating xylem zone (μm)	Average length of xylem fibre (μm)	Average length of vessel element (μm)
April	3–4	48 48 ± 5 81	1215 8 ± 27 4	–	1733 1 ± 154 5	1095 41 ± 42 3
May	4–5	64 75 ± 6 82	1264 6 ± 41 9	46 23 ± 4 87	1810 05 ± 126 5	1116 6 ± 72 4
June	6–7	90 09 ± 7 02	1290 7 ± 50 2	97 44 ± 5 51	1798 1 ± 169 2	1165 ± 72 3
July	7–8	103 14 ± 5 03	1284 9 ± 29 6	121 18 ± 7 49	1815 2 ± 111 7	1172 76 ± 55 6
August	8–9	96 25 ± 6 15	1327 40 ± 36 9	114 34 ± 8 02	1867 5 ± 125 1	1181 39 ± 85 07
September	7–8	93 60 ± 6 47	1283 30 ± 59 8	98 23 ± 6 37	1807 6 ± 147 4	1168 41 ± 103 9
October	6–7	87 70 ± 6 67	1266 03 ± 46 9	81 22 ± 5 63	1821 1 ± 146 5	1150 63 ± 88 1
November	5–6	67 77 ± 5 34	1248 10 ± 41 9	58 20 ± 4 46	1726 3 ± 134 06	1138 38 ± 79 8
December	4–5	54 56 ± 6 04	1203 1 ± 42 8	20 65 ± 2 28	1731 5 ± 131 01	1110 9 ± 83 2
January	3–4	41 98 ± 6 13	1168 1 ± 32 2	–	1653 4 ± 98 8	1011 30 ± 81 82
February	3–4	42 39 ± 5 13	1148 4 ± 33 2	–	1644 9 ± 103 7	1013 7 ± 96 1
March	3–4	45 27 ± 6 42	1197 04 ± 39 3	–	1746 07 ± 155 9	1073 39 ± 82 6

(–) no xylem production

(±) standard deviation

iodine-potassium iodide and Gibb's reagent, respectively (Johansen 1940, Gahan 1984 McCully 1966) All measurement were made on 100 samples of each of the wood elements The mean length and breadth of fusiform initial were calculated from slides in Transverse Longitudinal Section (TLS) of 100 randomly chosen fusiform initial Photographs were taken from Nikon E600 microscope

Data analysis

The mean value and standard deviation were calculated for all the measurement made Statistical analysis of relationship between climatic factors (monthly mean, maximum and minimum temperature, rainfall, relative humidity) and anatomical variables such as cambial zone width, length of fusiform initial width of differentiating xylem zone, length of xylem fibre and vessel element were calculated by using Karl Pearson's correlation coefficient Multiple (partial) regression analysis was done and (*t*) values were calculated to determine the influence of particular climatic factor on cambial activity and xylem production (Zar 1974, Schweingruber 1988)

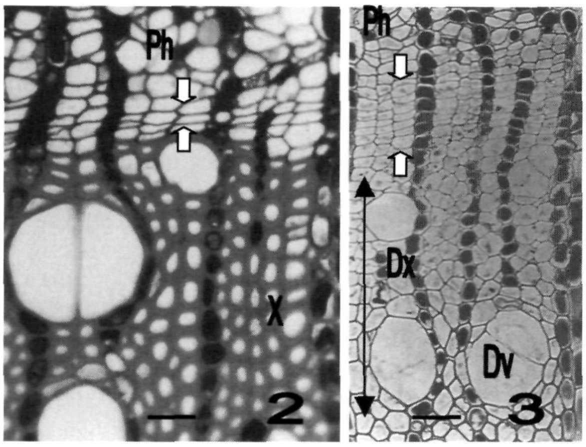
Results

Cambial activity in relation to phenology

The first visible indication of dormancy breakage in *D indica* was a slight swelling of young vegetative buds in the middle of April (pre monsoon or summer season), during which, though three to four cambial layers remained, the fusiform initial showed marked radial swelling By the middle of May, the maximum leaf flushing started and continued up to the end of December (end of pre-monsoon or summer season to the beginning of winter season) Sprouting of new leaves as well as persistent mature leaves were observed from middle of April to January (end of summer or pre-monsoon season to the middle of winter season) The number of cambial layers ranged from five to nine from May to December Consequently, the cambial zone width also increased (Table 1) Leaf senescence was initiated from the month of February, and the defoliation continued up to the end of March (winter to the pre monsoon season) The tree was completely barren for a brief period of 15 days, therefore, *D indica* belongs to brevideciduous type (Borchert 1999) During winter, the cambium consisted of only three to four layers and the average width of the cambial zone remained more or less the same (Table 1) Flowering was noticed in the months of June and July (monsoon season) Fruiting was observed in the month of August, and mature fruits were borne on tree from September to the last week of October The dispersal of seeds took place in the months of October–December (retreating monsoon period to the beginning of winter) (Fig 8)

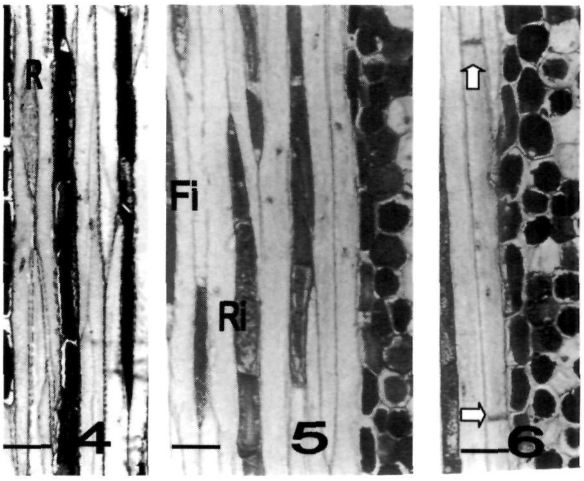
Cambial activity and xylem differentiation

In *D indica* L , the vascular cambium was non-storied with axially elongated fusiform initials and ray initials, the ray initial were unicellular, uniseriate with rectangular cells, while the multiseriate rays were comprised of more or less isodiametric cells The ray initials were filled with starch grains and phenolic contents (Figs 2–3) During the active period of vascular cambium in 8 months from the first week of May to the last week of December (pre-monsoon to towards the beginning of winter seasons), the cambial zone was wider consisting of five to nine cell layers (Fig 3) in each radial file During the months of July–September (monsoon season), cell walls of both the fusiform and the ray initials were



Figs. 2 and **3** Transverse sections of *Dillenia indica* stem showing the dormant and active vascular cambium respectively. Note the cambium is only three layers in the dormant period and six to eight layers in the active cambium (*white arrows*). Both the active and the dormant ray initials filled with phenolic contents (DX, differentiating xylem zone; DV, differentiating vessel elements; Ph, phloem; bar, 70 μm)

thin, and the beaded appearance of fusiform initials was not much prominent (Fig. 5). In contrast, during the dormant period of 4 months from January to the end of April (winter to the beginning of pre-monsoon seasons), the cambial zone was narrow, consisting of three to four layers only with relatively thick radial walls in transverse section and surrounded by mature xylem and phloem elements (Fig. 2). The initiation of cambial activity was marked by swelling of fusiform initials and the active vacuolation in the month of May (towards the end of pre-monsoon or summer season) and followed by periclinal divisions in the fusiform initials observed from May onwards. Consequently, the number of cells in the cambial zone and the width of cambial zone increased considerably. During the active period, the number of periclinal divisions was more than the number of anticlinal divisions (Fig. 6). The fusiform initials showed two to three nucleate and aseptate. The average data pertaining to the size of the cambial width zone, differentiating xylem zone, fusiform initial, xylem fibre and vessel element are shown for the different months of years 2002–2003 (Table 1). The cambial activity was slowed down from October to the end of December (from retreating monsoon to the beginning of winter season). In the months of November and December, the percentage of anticlinal divisions was more than the percentage of periclinal divisions. The cessation of cambial activity began towards the end of December, and dormancy was imposed from January to the end of April, and the tree was barren for a brief period of 15 days in April. The starch grains and protein bodies were masked by abundant phenolic contents in the ray initials during dormancy only, the raphids were noticed in the unicellular ray initial (Fig. 4) and on the phloem side; on the other hand, starch grains were more in the xylem rays and xylem parenchyma cells.



Figs. 4–6 Tangential longitudinal sections (TLS) of vascular cambium in *Dillenia indica*. **4**. Dormant period showing the beaded nature of fusiform initials, note the raphid needles are stored in the unicellular ray initials and phenolic contents in uni/multiseriate ray initials. **5** and **6**. Active vascular cambium showing the thin cell walls and beads are not prominent. Note the multinucleate condition of fusiform initials. The ray initials contain starch grains masked by phenolic contents. Periclinal division in the fusiform initials with phragmoblast (*white arrows*) (R, raphides; Ri, ray initials; Fi, fusiform initials; bar, 15 μm in **4** and **5**; bar 20 μm in **6**)

Differentiation of xylem tissue

The secondary xylem of *D. indica* L. consists of libriform fibres, vessel elements and axial parenchyma apotracheal diffuse and paratracheal vasicentric scanty. Details regarding the timing of initiation and the cessation of xylem production as well as its duration and the average width of the cambial zone and the average width of differentiating xylem zone are given (Table 1). Xylem production was noticed for a total period of about 8 months in *D. indica* L. with the formation of new fibres, vessel elements and xylem rays in the month of May after the formation of new foliage and branches; it was continued up to the last week of December. A comparison was made between the average length of fusiform initials with that of libriform fibres and vessel elements in different months of the year to find out the change in length, if any, during differentiation of fibres and vessel elements (Fig. 7B). The average length of xylem fibres and vessel elements showed the same trend of variation as that of fusiform initials in *D. indica* L.

Starch grains, polyphenol and tannin contents are the major reserved products found in the ray initials of cambium, xylem rays, and axial parenchyma cells and occasionally in the xylem fibres during dormancy (Figs. 2–3). During the onset of cambial activity, the starch grains were reduced in amount but not totally absent. Phenolic substances do exist in both the dormant and active periods of vascular cambium. The fibres produced during May were thin walled with larger lumen and angled in transverse section, the cell

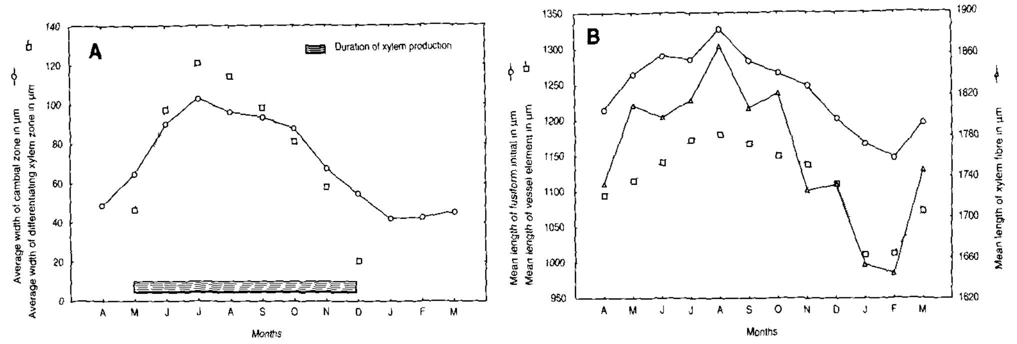


Fig. 7 Quantitative data of vascular cambium and its derivatives. **A** Showing the duration of xylem production (▬) width of cambial (– o –) and differentiating xylem zone (-□-). **B** Showing the mean length of fusiform initial (– o –), mean length of vessel element (-□-) and mean length of xylem fibre (–△–)

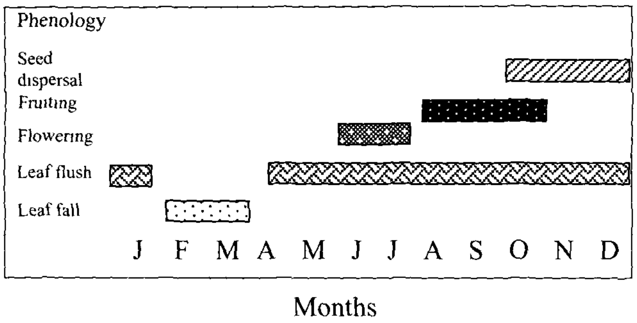


Fig. 8 *Showing the phenological diagram of D. indica during 2002–2003*

wall thickness ranged from 1.5 to 2 μm (Fig. 3); in contrast, the cell wall thickness of the late wood fibres produced during November ranged from 8 to 10 μm, and the fibres were radially compressed and the lumen was very narrow (Fig. 2). Similarly, the vessel elements produced during the active period had sclariform perforation plates with 50–60 bars, whereas those produced during dormancy had the sclariform perforation plate with 25–30 bars.

Relationship between climatic factors, cambial activity and xylem production

During the onset of cambial reactivation and the differentiation of xylem elements from the first week of May to the last week of December (pre-monsoon to the beginning of winter), a strong positive correlation was shown with monthly minimum ambient temperature and the correlation coefficients were very high (Table 2). The response function and the correlation coefficient of the cambial activity (including cambial zone width, length of fusiform initials) versus the monthly mean minimum temperature ($r = +0.85$, $r = +0.90$) was higher than the monthly mean temperature and the mean maximum temperature, respectively (Table 2). Similarly, the width of differentiating xylem zone, length of xylem fibres and vessel elements showed more positive correlation coefficient with the ambient monthly mean minimum temperature ($r = +0.88$, $r = +0.89$ and $r = +0.82$) than precipitation and relative humidity (Table 2).

The multiple (partial) regression analysis has shown that rainfall was statistically significant but had inverse relationship with the cambial zone width ($t \geq -2.29$), fusiform initials length ($t \geq -2.50$), and the differentiating xylem zone width ($t \geq -2.55$). The length of fibres did not show any relationship with any one of the climatic parameters because its t-value is less than 2.26. The length of vessel elements showed statistically significant positive correlation with monthly mean minimum ambient temperature (Table 2). Other climatic parameters were not statistically significant with any one of the quantitative data of the vascular cambium and its derivatives. However, among the mean maximum, mean minimum and mean temperature, the B-value of the mean minimum temperature showed higher value than the rest. Therefore, the mean minimum temperature had some effect on the cambial activity and wood formation. Moreover, during the onset of the cambial reactivation and differentiation of xylem elements during first week of May to the last week of December (pre-monsoon to the beginning of winter season), a positive correlation was shown with monthly minimum ambient air temperature and the correlation coefficients are very high (Table 2). The effect of rainfall and relative humidity on both the cambial activity and differentiations of xylem elements was secondary in nature.

Discussion

The cambial reactivation and xylem differentiation were seen in the month of May (pre-monsoon or summer season), 2 weeks after the onset of bud breaking during the middle of April in *D. indica* L. at this study site (Fig. 7A; Table 1). The same feature was observed in the sub-tropical climate plants like, *Pyrus communis* L. and *Pyrus malus* L. (Rosaceae) (Evert 1961, 1963b), *Pinus strobus* L. (Pinaceae) (Murmanis 1971) and *Quercus boissieri* Reut. (Fagaceae), *Pistacia alantica* Desf. (Anacardiaceae) (Fahn and Werker 1990), as well as the tropical species *Tectona grandis* L.f (Rao and Dave 1981; Venugopal and Krishnamurthy 1987; Priya and Bhat 1999; Rao and Rajput 2001a, b). Reactivation of the cambium in different months in the same species

Table 2 Correlation coefficient (r) and (t) values of cambium and its derivatives versus different climatic factors

Anatomical variables	Climatic factors									
	Monthly mean temperature (°C)		Monthly mean maximum temperature (°C)		Monthly mean minimum temperature (°C)		Monthly mean precipitation (mm)		Monthly mean relative humidity (%)	
	r	t	r	t	r	t	r	t	r	t
Average width of cambial width zone (μm)	+0.81	−1.09	+0.73	−1.31	+0.85	+2.09	+0.78	−2.92*	+0.75	−0.39
Average length of fusiform initial (μm)	+0.87	−0.41	+0.81	−1.61	+0.90	+1.61	+0.78	−2.50*	+0.80	−0.87
Average width of differentiating xylem zone (μm)	+0.87	−0.71	+0.84	−1.18	+0.88	+1.64*	+0.72	−2.55	+0.69	+0.48
Average length of xylem fibre (μm)	+0.88	−1.42	+0.85	−0.21	+0.89	+1.98	+0.70	−2.16	+0.70	−0.94
Average length of vessel element (μm)	+0.80	−1.34	+0.74	−1.35	+0.82	+2.45*	+0.66	−2.29	+0.64	−1.79

All r values are significant at $p < 0.05$ levels in *Dellinia indica*

*t-values are significant at $p < 0.05$

growing under different local climatic condition is reported in some evergreen species (Zimmerman and Brown 1971), but such comparative studies for the sub-tropical wet forest are lacking.

The cambial activity continues for 8 months from May to December (pre-monsoon to the beginning of winter season), and dormancy from January to the end of April (winter to the pre-monsoon or summer seasons) is imposed strongly by climatic condition. The leaf fall started from February, and complete defoliation resulted in the tree being barren for a brief period of first 15 days during the month of April. Therefore, *D. indica* L. growing in the sub-tropical climate of northeast India belongs to brevideciduous (William et al. 1997, Borchert 1999). It simulates the phenology of tropical deciduous tree (Philipson et al. 1971, Dave and Rao 1982, Fahn 1982, Liphschitz and Lev-Yadun 1986, Venugopal and Krishnamurthy 1987, Rao and Rajput 2001a, b).

Though *D. indica* L. growing in the sub-tropical wet climate within a limited habitat shows distinct annual rhythm in the cambial activity, it results in the formation of invisible growth ring annually (Carlquist 1980, Liphschitz et al. 1981, Fahn et al. 1968). In *Quercus costaricensis* Liebm. (Fagaceae), also growing in mountain sites in Costa Rica, no clear rings with annual periodicity (Worbes and Junk 1989) are formed. Brevideciduous species are confined to microsites with adequate soil water (Borchert 1994, Nepstad et al. 1994). In *D. indica* L., xylem production was seen only once in a year, i.e. from May to the last week of December. A similar pattern of xylem production is seen in most of the temperate and tropical trees (Philipson et al. 1971, Fritts 1976, Larson 1994, Iqbal 1994, Schweingruber 1996, Worbes 1995, 2002). However, for some trees growing in Israel (Fahn 1958, Liphschitz et al. 1981), Nigeria (Amobi 1974) and peninsular India (Venugopal and Krishnamurthy 1987), two flushes of xylem production in a year are reported. Various anatomical features such as the presences of terminal parenchyma, initial parenchyma, radially compressed fibres and a lower frequency of vessel in the latewood are used to demarcate the growth ring in tropical wood (Chowdhury 1964, Carlquist 1980). In *D. indica* L., tangentially compressed two to three layers of fibres demarcate the growth ring (Pearson and Brown 1981). In temperate plants over winter, partially differentiated xylem element has been reported in few plants (see also Romberger 1963, Longman and Coutts 1974) in *Quercus* species, (Timell 1980) in *Picea abies* L. (Pinaceae), *Quercus rubra* L. (Fagaceae) (Bannan 1955) in *Thuja occidentalis* L. (Pinaceae). Such phenomenon has not been encountered in *D. indica* L.

Shorter element characterized the end of xylem production, while the longer element marked the peak period of the cambial activity and xylem production. Simultaneously, the diameter of wood element was greater during the period when their production was at its peak and minimum during the period when the activity of cambium was the lowest An analysis of earlier literature reveals that this trend is shown by the largest number of plants studied (see also Taylor 1976), even though the presence of longer late wood fibres has been recorded in certain temperate trees (Bisset and Dadswell 1950, Panshin and De Zeeuw 1980)

Probably starch and crystal of calcium formed the source material for the new cell wall synthesis (e g carbohydrates and calcium pectate) when the cambial derivatives are rapidly produced The maximal and minimal starch content in the xylem of *Abies balsamea* (L) Mill (Abietaceae) associated respectively with the period of cambial dormancy and reactivation (Parker 1960, Sauter 1966, Pomeroy and Siminovitch 1971, Tsuda and Shimaji 1971, see also Riding and Little 1984, Essiamah and Eschrich 1985) When there are two flushes of cambial activity and dormancy, the accumulation and depletion of starch and calcium took place twice a year (Venugopal and Krishnamurthy 1987)

The timing of reactivation, peak activity of cambium and xylem production and mean cell length in *D indica* L were studied in relation to the variation in the climatic factors such as temperature, relative humidity and precipitation Periodicity of cambium and xylem production is controlled by various environmental and physiological factors (Kramer and Kozlowski 1979, Venugopal 1986, Ajmal and Iqbal 1987, Larson 1994, Rao and Rajput 1999) The monthly mean values of the climatic factors mentioned earlier for all the month of the years 2002 and 2003 are presented (Fig 1A and B) It was observed that both the reactivation and the peak activity of cambium and xylem production were generally favoured by the mean minimum temperature (15–21°C) in *D indica* Linn The tree showed a positive correlation between the lowest cambial activity and the absence of xylem production with the lowest temperature range (5–12°C) during January–April Therefore, a rise of 6°C is enough to reactivate cambium as well as induce new buds and foliage in *D indica* L after dormancy Higher temperature was reported to be conducive for cambial reactivation and xylem production in *Picea glauca* (Monech) Voss (Pinaceae) (Gregory and Wilson 1968) A similar view was expressed by Kramer and Kolzowski (1979) that temperature was a significant factor for bud break following reactivation and subsequent shoot growth On the other hand, a rise and fall in temperature was reported to have no effect on the cambial activity in *Eucalyptus camaldulensis* Dehn (Myrtaceae) (Waisel et al 1966) and in *Cupressus sempervirens* L (Cupressaceae) (Liphschitz et al 1981) It appears that temperature factor does not act independently and the law of limiting factor may be in operation (Coile 1936, Keen 1937) The mechanism by which higher temperature promoted the cambial reactivation in many trees is not clear On the basis of in vitro experiment, the increase in temperature was responsible for the release of auxin reserve from the tissue adjacent to cambium, which, in turn, activated cambium (Wort 1962) On the other hand, temperature had implicated in promoting vacuolation of fusiform initial and may likely be the effect of increased temperature (Catesson 1962) The role of rainfall on cambial behaviour and xylem production under drought condition was studied much more intensively than other factor in the past Higher rainfall was reported to be conducive to cambial reactivation in several plants growing especially in the tropic and the semi-arid climates (Glock 1955, Reinders-Gouwentek 1965, Roger 1981, Dave and Rao 1982) The present study also shows that the mean rainfall has less correlation than mean minimum temperature in *D indica* L (Table 2) Rainfall probably is an important factor only in the regions where the soil moisture content is dependent on rainfall (Rao and Rajput 2000, 2001a, b) This study has indicated that cambial reactivation, peak activity and xylem production were not limited by rainfall because *D indica* L grows only in limited habitat of sub-tropical wet forest of northeast India, with enough moisture throughout the year In other words, *D indica* is not subjected to physical drought or water stress (Fahn 1959a, b, Amobi 1974) However, the monthly mean precipitation is below 50 mm during December–February (winter season) and the soil type is oxisols where moisture content is 30–40% (Pandey 2004, Porwal et al 2000, Tripathi 2002, Brady and Well 2002) Moreover, this study site is located 60 km from Cherrapunji, which is the region of highest rainfall (965 cm per year) in the world (Lal 2000, Anonymous 1971, 1972) It was observed that a higher mean minimum temperature generally favoured both cambial reactivation and xylem production in *D indica* L The other factors such as relative humidity and precipitation have little effect on the cambial periodicity and xylem differentiation In *Pinus kesiya* Royle ex Gordon (Pinaceae), *Cedrus deodara* Loudon (Pinaceae), *Cryptomeria japonica* D Don (Cupressaceae) growing in Meghalaya at an altitude of 1,500 m a s l , mean temperature (15–22°C) played an important role in cambial reactivation and xylem production (Dhirendra Singh 2002) But *D indica* L growing at an altitude of 1,100 m a s l showed response only to mean minimum temperature The difference of an altitude of 400 m played an important role in the reactivation of cambium and xylem production It was interesting to observe that the mean temperature at 1,500 m a s l (upper Shillong) is equal to the mean minimum temperature at 1,100 m a s l (15–22°C) at the Botanical Survey of India, Experimental Garden, Shillong Present study on *D indica* growing in the sub-tropical wet forest in the north-eastern hilly region indicate that the trees develop adaptive strategies in response to the altitude and local climate

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