

Title	Growth ring formation of selected tropical rainforest trees in Peninsular Malaysia(Dissertation_全文)
Author(s)	Amir Affan Abdul Azim
Citation	Kyoto University (京都大学)
Issue Date	2014-03-24
URL	http://dx.doi.org/10.14989/doctor.k18338
Right	学位規則第9条第2項により要約公開; 許諾条件により本文は2015-04-01に公開
Type	Thesis or Dissertation
Textversion	ETD

Growth ring formation of selected tropical rainforest trees in
Peninsular Malaysia

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2014

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Abbreviations

AHFR	Ayer Hitam Forest Reserve
BHFRIM	Bukit Hari, Forest Research Institute Malaysia
MAFR	Mata Ayer Forest Reserve

Chapter 1

AD	anticlinal division or just after division
CC	callus cells
CSWV	completed secondary wall vessel
CT	callus tissue
CZ	cambial zone
EVE	enlarging vessel element
EZ	enlarging zone
FC	radially flattened cells
NFVE	newly-formed vessel element
NW	normal wood
SV	the layer included with small vessel formed after marking
TRC	traumatic resin canal
TRWC	thick radial wall of flattened cells
TVE	thickened-wall vessel element

Chapter 2

AP	axial parenchyma
AR	ambiguous growth ring
DC	discontinuous growth ring
EC	epithelial cell
F	fiber
FZ	fiber zone
IT	intermittent growth ring
MP	marginal parenchyma
RC	resin canal
RTC	occurrence of resin and/or traumatic canal
TF	thick-walled fiber
TRC	traumatic resin canal
VSD	variation in vessel size and/or density

Chapter 3

CC	Crushed cells
CD	cell deposits
DC	direct current
ER	extended ray parenchyma
TRC	traumatic resin canal

General Introduction

Climate change, forest degradation and demand for wood products stimulate the practice of sustainable forest management in recent years. However, forests, plantations and wood supplies can be sustainably managed with certainty when there is information of tree growth and wood formation available. In Peninsular Malaysia, selective management system (SMS) was applied for stand development to overcome accelerated forest degradation and decline to realize sufficient wood supply for timber industries. Based on given values of stem diameter at breast height (d.b.h) set for dipterocarp and non-dipterocarp groups, trees were separated into size classes such as seed producer, harvest-available and future-harvest. However, the regulation established in SMS was somehow arbitrary because trees react physiologically different with ecological differences, thus occurring problems remain unsolved.

One of the ways to acquire knowledge on tree growth and wood formation are through tree-ring analysis. In the tropical region, lack of visible growth rings in trees causes limitation to practice dendrochronology, evaluate tree growth rate, and estimate tree age. Although it is thought that most tropical rainforest trees do not form annually distinct growth rings, preliminary observation of some trees in Peninsular Malaysia shows that they have “ring-like structure” even if it is very weak or faint. Therefore, the “ring-like structure” in tropical rainforest trees becomes an important target to be investigated.

Worldwide interest in tropical dendrochronology has been increasing. Rozendaal and Zuidema (2011) and Pumijumnong (2013) reviewed the potential of tree-ring analysis in the tropics. The development of tree-ring chronologies of *Tectona grandis* in India (e.g., Bhattacharyya et al. 1992, Borgaonkar et al. 2007), Java (e.g., D'Arrigo et al. 1994), Thailand (e.g., Pumijumnong et al. 1995), and Myanmar (e.g., Pumijumnong et al. 2001) has stimulated interest on formation of growth ring in other tropical species. Few applications in tropical seasonal forests (e.g., Brienens et al. 2009) and mangrove forests (e.g., Menezes et al. 2003, Chowdhury et al. 2008) showed that tropical dendrochronology can be applied. Limited studies have been conducted in tropical rainforest (e.g., Fichtler et al. 2003, Soliz-Gamboa et al. 2011, Lopez et al. 2012), because most tropical rainforest trees were thought not to produce anatomically distinct annual growth rings.

Worbes (1995, 2002) reviewed several methods which can be applied to investigate annual rhythm of radial growth in tropical trees, and categorized them as direct measurement of radial growth and detection of changes in wood formation. Radial growth can be estimated by dendrometer measurement (e.g. Ohashi et al. 2001, Baker et al. 2002, da Silva et al. 2002), cambial marking (e.g. Shiokura 1989, Fujiwara 1992, Nobuchi et al. 1995, Sass et al. 1995) and cambial zone monitoring (e.g. Venugopal and Krishnamurthy 1994, Rao and Rajput 1999). Changes in wood formation can be detected through anatomical (e.g. Brienen et al. 2009, Ohashi et al. 2013) and isotopic analyses (e.g. Poussart et al. 2004, Verheyden et al. 2004a, Verheyden et al. 2004b, Ohashi et al. 2009).

Dendrometer enables continuous measurement of diameter increment and provides information on growth rhythm. However, the increment measured by dendrometer includes the phenomena occurred in a bark, therefore the results are partially reliable to evaluate the growth rhythm. Wood anatomical changes in a certain period can be pursued through cambial markings in which visible marks are created in the xylem tissue. The fundamental knowledge of wood formation in a certain period can be understood through anatomical observation of cambium and cells derived from it. However, periodic sampling of green phloem-xylem blocks on a stem of single tree and delicately prepared samples for microscopy are required to investigate the rhythm of cambial activity.

Some studies reported visible growth ring as well as invisible growth indicator in tropical trees. The former were investigated through anatomical studies (e.g. Worbes 1985, Roig et al. 2005, Maingi 2006) and the latter by isotope analyses (e.g. Poussart et al. 2004, Verheyden et al. 2004a, Ohashi et al. 2009). Worbes (1985) classified tree-ring types appeared in tropical trees as 1) density variations (fibres), 2) terminal parenchyma bands, 3) a repeated pattern of alternating fiber and parenchyma bands, and 4) variation in vessel distribution and/or size. In some occasion, the mentioned anatomical features can occur in various combinations. Unfortunately, those features are restricted to particular species and areas with obvious seasonality in climate. The variation of stable isotope concentrations in tree rings of temperate zones was traced to variations in climate (Long 1982) to the ringless species (e.g. Poussart et al. 2004, Verheyden et al. 2004). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in xylem record climate-related variables such as precipitation (Saurer et al. 1997), temperature and irradiance (McCarroll and Loader 2004). However, there are some uncertainties in the results because

isotope discrimination in plants are complex and not yet fully understood. Besides, the measurement of stable isotope content in xylem is expensive and time consuming which limit the application to long- and high-resolution chronology.

Regardless of the occurrence of faint or weak “ring-like structures” in some tropical rainforest trees in Peninsular Malaysia, it is important to clarify the rhythm of radial growth and detect the occurrence of growth rings in tropical rainforest trees. In the present study, the author aimed to clarify the annual formation of growth rings in tropical rainforest trees. Thus, to accomplish the main goal, it is important to (1) investigate the occurrence and anatomical features of visible growth rings, (2) apply effective methods, (3) determine the annual rhythm of radial growth, and (4) verify the annual formation of visible growth rings in tropical rainforest trees in Peninsular Malaysia.

In Chapter 1, dendrometer measurement, light microscopy of cambium, cambial marking, and phenological observation were applied and tested in selected tropical rainforest trees in Peninsular Malaysia. Through this study, the author found that some of the selected tropical rainforest do have ring-like structures, seasonal radial growth, cambial activity and leaf phenology. The author also proposed for improvement of methods and application of isotopic analysis to enhance the study of wood formation in tropical rainforest trees. Chapter 2 discussed on the occurrence and anatomical features of growth rings in selected tropical rainforest trees, classified as group of well-defined, poorly-defined and absent growth rings. Based on the results discussed in Chapter 1, Chapter 3 and 4 discussed the improvement and additional methods. The application and effectiveness of high direct current (DC) pulse cambial marking and stable carbon isotope ($\delta^{13}\text{C}$) measurement in tropical rainforest trees were discussed in Chapter 3. Chapter 4 discussed the rhythm of radial growth and cambial activity based on measurement using dendrometer and electric impedance, coupled with phenological observation. Annual formation of growth ring in species belongs to the group of well-defined growth rings, estimated through the synchronicity among anatomical changes, variation in $\delta^{13}\text{C}$ values, annual rhythm of radial growth, cambial activity, leaf phenology, and precipitation, were discussed in Chapter 5.

Chapter 1

Characteristics of Wood Formation in Tropical Rainforest Trees in Peninsular Malaysia

1.1 Introduction

Seasonal characteristics of cambial activity in temperate regions can be easily traced by the variation in climate, such as day-length and temperature. However, because tropical climates are often regarded as non-seasonal, there is much uncertainty as to the factors controlling cambial activity under tropical conditions (Alvim 1964). There were some studies reporting seasonal characteristics of vascular cambium in the tropics (e.g. Rao and Rajput 2001, Pumijumnong and Wanyaphet 2006). In order to understand the characteristics of wood formation in tropical rainforest trees, it is important to investigate the cambium.

Among common and useful approaches and methods for investigating xylem formation in trees are direct measurement of radial growth through dendrometer and anatomical approaches through cambial observation and cambial marking. Annual rhythm of wood formation can be well examined with independent factors of leaf phenology and meteorological conditions. Cambial marking is applied to investigate wood formation during a recognized duration. The ‘pinning method’, invented by Wolter (1968) became a useful tool for this objective. Shiokura (1989) modified the pinning method by using a nail instead of tiny pin. Fujiwara (1992) introduced the ‘knife-cutting injury’ method by using a knife. Most of the methods were applied to temperate trees and some for tropical trees.

To ensure that the study of xylem formation can be investigated, it is important to identify the cambial response on marking and exact position of marked cambium. Denne (1977), Yoshimura *et al.* (1981a & 1981b) and Kuroda and Shimaji (1984) had investigated the effect of wound to the cambial differentiation. There were some references to the position of marked cambium and cambial cells, (1) series of traumatic resin canals (Kuroda and Shimaji 1983) and (2) region with a small vessels and increased of cell rows in tangential direction (Nobuchi *et al.* 1995).

This study investigated the seasonal characteristics of cambial activity and cambial response to marking, and deduced the anatomical features of marked cambial zone. Through this study, the

effectiveness of cambial observation and cambial marking for the study of wood formation in tropical rainforest trees were discussed.

1.2 Materials and Methods

1.2.1 Study sites and species selection

This study was conducted in two forest plantations managed by Forest Research Institute Malaysia (Fig. 1.1), located at Mata Ayer Forest Reserve, Perlis (MAFR, 6°40'N, 100°15'E) and Bukit Hari, Forest Research Institute Malaysia, Selangor (BHFRIM, 3°14'N, 101°38'E). The climate of MAFR is classified as tropical monsoon (A_m according to Köppen-Geiger climate classification, Peel et al. 2007), with a 2–3-months of dry season from December to February. The climate of BHFRIM is classified as tropical rainforest (A_f according to Köppen-Geiger climate classification), in which the monthly precipitation is usually much greater than 100 mm.

Five experimental trees of *Azadirachta excelsa*, *Hopea odorata*, and *Khaya ivorensis* grown in MAFR and BHFRIM were selected for this study. In MAFR, *A. excelsa* (hereafter this is called as AEMA) was planted close to the river in 1996. *H. odorata* (HOMA) was planted close to small stream in 2000. *K. Ivorensis* (KIMA) was planted in 2000 and located far from any water source. In BHFRIM, *A. excelsa* (hereafter this is called as AEBH) was planted in 1997 on a hill. *H. odorata* (HOBH) was planted in 1997 together with *Dyera costulata*, and located at the downhill site. *K. Ivorensis* (KIBH) was planted in 1998 together with *A. excelsa*, located at an uphill location.

1.2.2 Monitoring of diameter growth and observation of leaf phenology

Annual growth of stem radii for each tree species were monitored with dendrometers. Phenomena of leaf changes were observed by using a binocular. Dendrometer measurement and phenological observation were performed once a month from May 2008 to June 2009. Scale of 0 (0%), 1 (less than 20%), 2 (in between 20–80%), and 3 (more than 80%) were used in recording the cover percentage of mature leaves (dark green leaves), shed leaves, leaves emergence (new shoots), and fully expanded leaves (leaves fully expanded from shoots but light-green in color) on tree crown. In

this study, the records of dendrometer readings and leaf phenology were used to relate and discuss the seasonality of cambial activity in selected tropical rainforest trees.

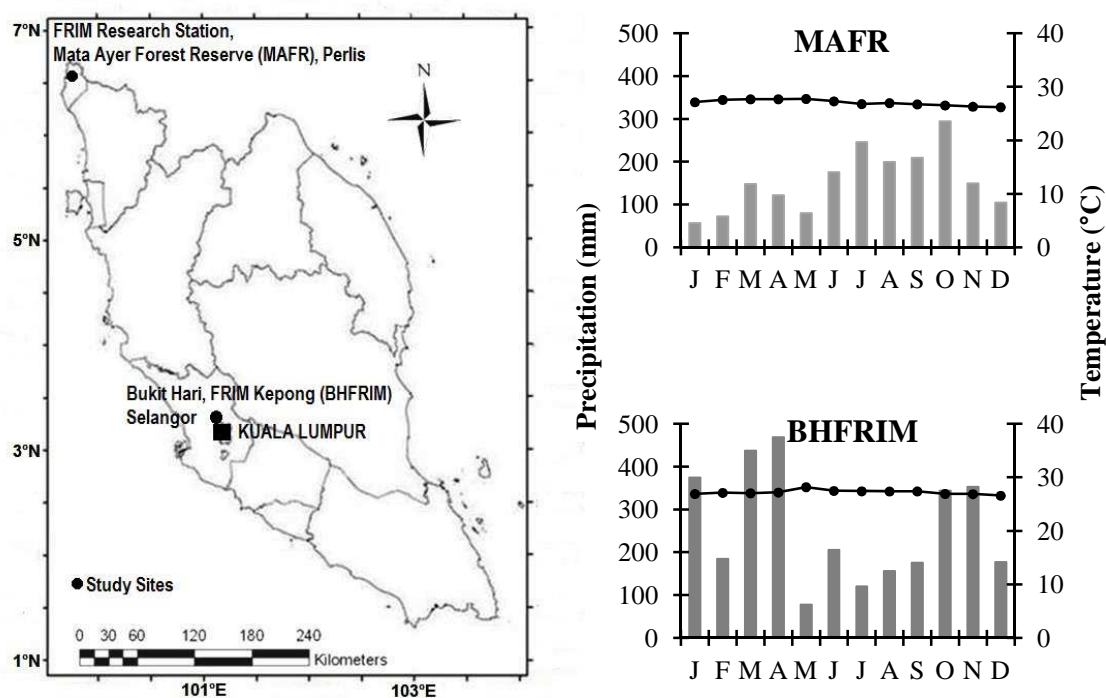


Fig. 1.1 Location of research sites and climate diagram for each area. Monthly precipitation (bar) and temperature (line) are the mean from 2000 to 2007. Climate data for the Mata Ayer Forest Reserve (MAFR) and Bukit Hari, Forest Research Institute Malaysia (BHFRIM) were obtained from the Malaysian Meteorological Department.

1.2.3 Cambial observation

Small wood block including bark was collected every three months (August 2008, November 2008, February 2009, and May 2009) using chisel and handsaw. The extracted wood block was chemically fixed with 3% glutaraldehyde (GA) for light microscopy.

GA-fixed wood blocks were washed with water and cut into small wood blocks with size of 2 mm (T) × 7 mm (R) × 10 mm (L). Small wood blocks were dehydrated with a series of ethyl alcohol (EtOH). After small wood blocks were dehydrated in absolute EtOH, they were replaced with epoxy resin using propylene oxide as the intermediate. The wood blocks were transferred into a plastic box with epoxy resin and polymerized by heating in an oven at 37°C for 24 hours, 45°C for 24 hours and 58°C for 24 hours. Epoxy-embedded wood blocks were cut with a handsaw and trimmed using a blade to expose the desired surface of the block. After immersed the epoxy-embedded block in water, transverse sections with a thickness of 10-20 μm were cut using a sliding microtome.

Periodic Acid-Schiff's (PAS) reaction (McManus, 1948) was applied as the staining method to clearly reveal the fine structure of un lignified cambial cells. Cambial and enlarging zone cells were observed and identified using a polarized and bright light under a Leica Leitz DMRB compound microscope. The number of cambial zone and enlarging zone cells were counted to characterize the seasonality of cambial activity.

1.2.4 Cambial zone cells, enlarging zone cells and terminology

Cambium consists of a single cell layer surrounding a tree between the bark and the wood. In a radial cell file from the bark to the xylem, only one cell is theoretically the cambial initial. In reality, it is not easy to judge the cambial initial in a transverse section. This is because some cells adjacent to the cambial initials have the ability to divide and showing a similar shape as cambial initials. The term “cambial zone” is common for the group of cells including cambial initials and cells with the ability to divide (Schmid 1976). All of the radially flattened cells which were located in a region between phloem and xylem were judged to be in periclinal division or showing such pattern were counted as cambial zone (CZ) cells (Plate 1.1).

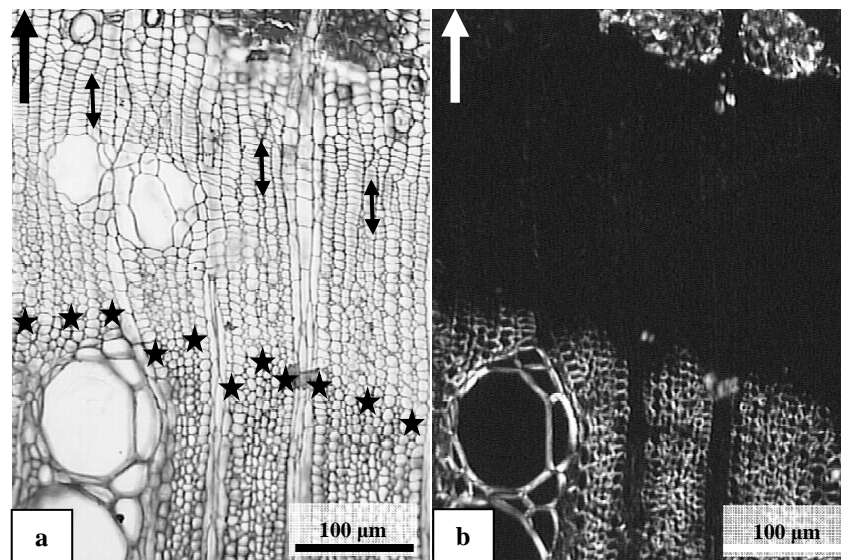


Plate 1.1 Transverse sections of *Azadirachta excelsa* showing cambial zone cells and enlarging zone cells. **(a)** Conventional light micrograph **(b)** Polarized light micrograph. Double-headed arrows indicate cambial zone and stars indicate the initiation of S_1 layer formation. Arrows indicate the direction of the bark side.

The process of cell completion involves cell division, enlargement and thickening of cell wall. Beyond cambial zone, each cell starts to enlarge and radially expand. In a transverse section, those

enlarging zone cells increase in radial diameter and show the pattern of intrusive growth. The cessation of enlargement is the start of secondary wall formation that can be observed under a polarized light microscope (Plate 1.1b), because cells with secondary wall reflect bright light. The last or final enlarging zone cell is a cell just before the secondary wall layer (S_1) forming cells. For the enlarging zone cells, total number of cells from those next to CZ to the innermost enlarging zone (EZ) cells was counted.

When counting cell number, it is important to investigate the effect of vessel formation because cells differentiating into vessel elements enlarge conspicuously and occupy a wide space in the enlarging zone. Wakuta *et al.* (1973) reported that radial cell rows having vessel element start to enlarge earlier than cell rows without vessel. CZ and EZ cells were counted in rows with and without the vessels to identify the cambial activity. The average numbers of CZ and EZ cells in rows with and without the vessels were checked with precipitation data to investigate the seasonality of cambial activity.

In this study, the author categorized the cambial activity as active and less active cambium. Active cambium was termed when there was a pattern of anticlinal division or just after the division (AD), newly-formed (NFVE) or enlarged vessel element (EVE), and the number of CZ and EZ cells was high and increased. Less active cambium was termed when there was no pattern of AD, no NFVE, had thickened-wall vessel element (TVE), and the number of CZ and EZ cells was less or decreased. In some occasions, thick radial wall of flattened cells (TRWC) can be observed in those featuring less active cambium.

1.2.5 *Cambial marking*

In this study, cambial marking was performed to investigate the cambial response on marking, deduced the marked cambial zone in selected tropical rainforest trees, and determined the appearance of ring-like features in selected tree species. The applicability and suitability of cambial marking in tropical rainforest trees were also discussed to examine whether the study of wood formation in tropical rainforest trees can be carried out precisely in the future.

Marking of cambium was performed every two months from June 2008 to April 2009 using a knife. The marking positions were arranged in zigzag pattern for all species with distances of 4 cm in tangential and longitudinal direction between knife-marks. Wood block samples including the knife-mark were collected from the wood disks for anatomical observation. Transverse sections 20–30 μm in thickness were cut using a sliding microtome and double-stained with safranin and fast green. All transverse sections were observed microscopically under Leica Leitz DMRB compound microscope.

1.3 Results and Discussion

1.3.1 *Anatomical features of ring-like structures*

In this study, the author used the term “ring-like structure” as a preliminary term before it can be judged as the growth ring. The main reason was because it is not yet clarified whether tropical rainforest trees, especially in Peninsular Malaysia, do form growth rings or not.

Ring-like structures were observed in all studied species. AEMA showed obvious ring boundaries, featuring dark and light colored bands on the transverse plane of the wood disk (Plate 1.2a). Microscopically, the darker bands were characterized by thick-walled fibers with small radial diameter; the lighter bands were featured by axial parenchyma (Plate 1.2b). AEBH also showed a similar ring-like structure even if it is not as obvious as AEMA. HOMA and HOBH did not show ring-like structures. KIMA and KIBH showed ring-like structures featured by darker bands on the transverse surface of the wood disk (Plate 1.3a). Microscopically, the darker band was characterized by thick-walled fibers (Plate 1.3b).

Through general observation of the studied species, it was considered that some of tropical rainforest trees do have ring-like structure, even if it is weak or faint. Identification and clarification of discovered ring-like structures became one of the main targets to be examined in this study, through investigating the seasonality of radial growth and cambial activity, and perhaps direct observation of the ring-like structure formation through cambial marking during one-year experimental period.

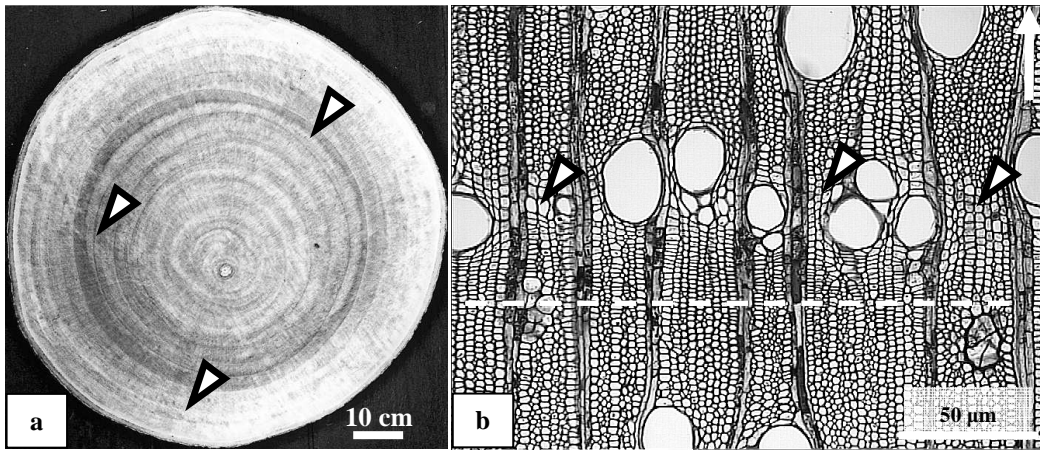


Plate 1.2 Transverse surface and section of *Azadirachta excelsa* showing ring-like structure. **(a)** Arrowheads on transverse surface indicate light colored bands featuring the ring-like structure. **(b)** Transverse section showing a band of axial parenchyma (arrowheads). Dashed line indicates the boundary of fiber changes from large to thin radial diameter and thin- to thick-walled fibers. Arrow indicates the direction of the bark side.

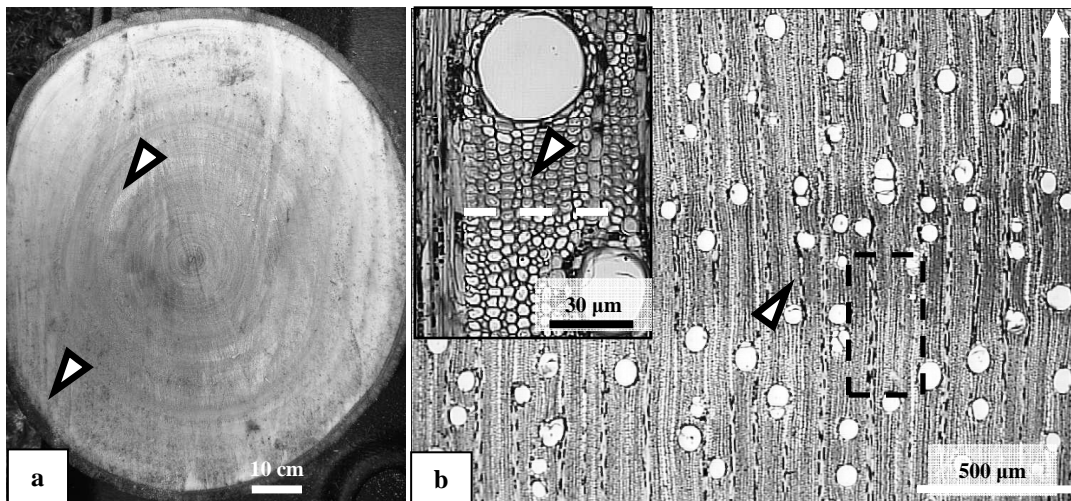


Plate 1.3 Transverse surface and section of *Khaya ivorensis* grown in Bukit Hari FRIM showing ring-like structure. **(a)** Arrowheads on wood disk indicate dark colored band featuring ring-like structure. **(b)** Transverse section showing band of fibrous zone (arrowhead). Inset image shows the enlargement (from dashed box) of fiber changes from thin- to thick-walled fibers (dashed line). Arrow indicates the direction of the bark side.

1.3.2 Radial growth and leaf phenology

AEMA showed a seasonal rhythm of radial growth in all five individuals (Fig. 1.2a). All individuals showed active radial growth from May 2008 to December 2008. During this period, AEMA grew in the rainy period and all individuals were fully covered with leaves (Fig. 1.2c). AEMA slowed down radial growth from January to February 2009. During this period, AEMA experienced a dry period, shedding of leaves and leafless condition in January 2009. In March 2009, all individuals restarted radial growth and continued until June 2009 when all trees flushed new leaves and grew in a rainy period. In BHFRIM, AEBH showed continuous radial growth (Fig. 1.2.b), with always

receiving much amount of precipitation throughout a year. Different from AEMA, AEBH did not become leafless even though they shed their leaves (Fig. 1.2d). Based on the differences between AEMA and AEBH, the author characterized AEMA as having seasonal radial growth while AEBH showed continuous radial growth.

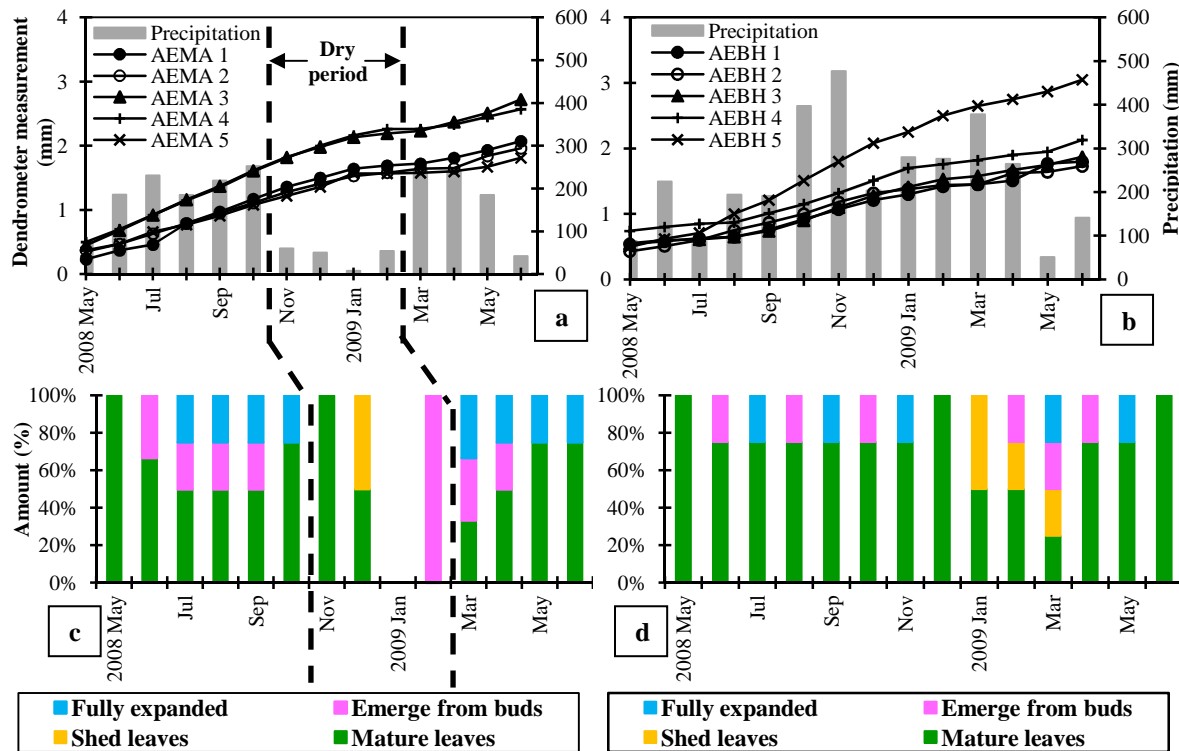


Fig. 1.2 Dendrometer measurement (**a and b**) and leaf phenology (**c and d**) of *Azadirachta excelsa* grown in Mata Ayer Forest Reserve (MAFR, AEMA) and Bukit Hari FRIM (BHFRIM, AEBH). (**a and c**) AEMA. (**b and d**) AEBH. Dashed lines were the estimated relationships of diameter increment with precipitation and leaf phenology in AEMA. Precipitation data for MAFR and BHFRIM were obtained from the Malaysian Meteorological Department.

Evergreen HOMA showed seasonal rhythm of radial growth in all five individuals (Fig. 1.3a). All trees showed active radial growth from May to October 2008. During this period, HOMA grew in the rainy period and all individuals were flushing new leaves (Fig.1.3c). HOMA slowed down radial growth from November 2008 to February 2009. During this period, HOMA experienced drought but leaves remained intact on the tree until the end of the dry period. All individuals restarted radial growth in between March and June 2009 when they experienced a rainy period. In BHFRIM, all HOBH trees showed continuous radial growth (Fig. 1.3b). Trees were always covered with leaves (Fig. 1.3d) and always received a lot of precipitation throughout the experimental period. Based on the differences between HOMA and HOBH, the evergreen HOMA can be characterized as having a seasonal radial growth whereas HOBH showed a continuous growth.

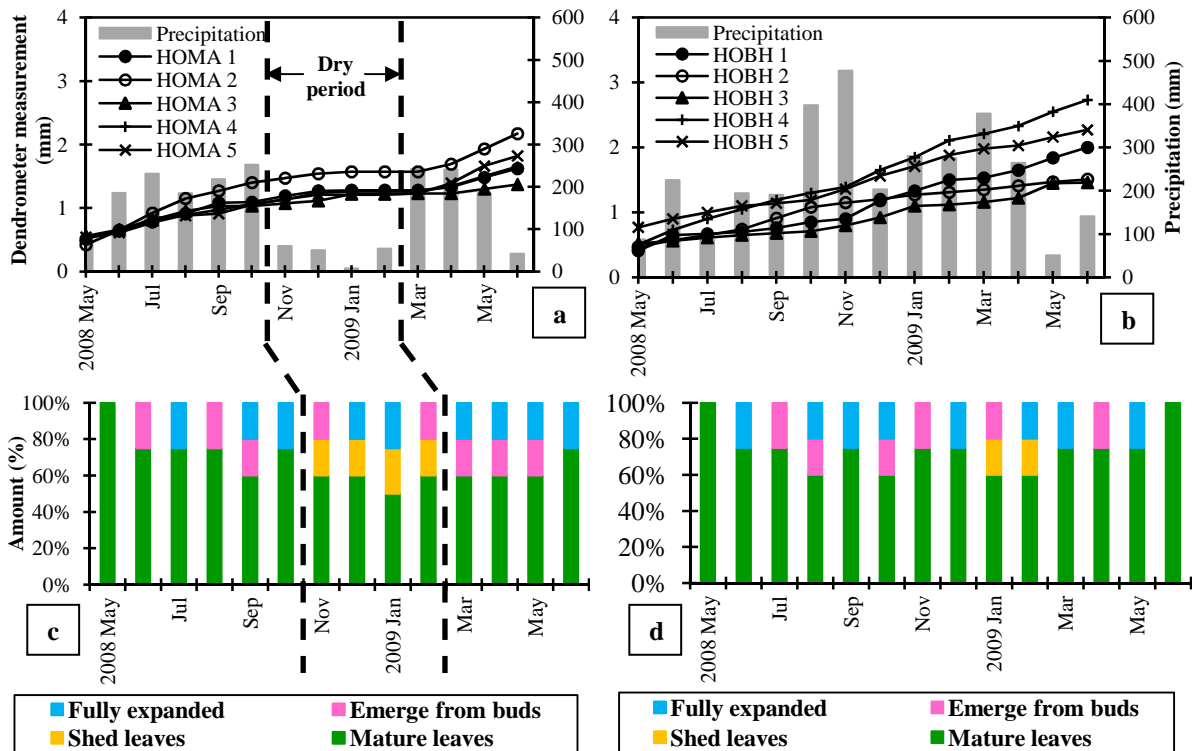


Fig. 1.3 Dendrometer measurement (**a and b**) and leaf phenology (**c and d**) of *Hopea odorata* grown in Mata Ayer Forest Reserve (MAFR, HOMA) and Bukit Hari FRIM (BHFRIM, HOBH). (**a and c**) HOMA. (**b and d**) HOBH. Dashed lines were the estimated relationships of diameter increment with precipitation and leaf phenology in HOMA. Precipitation data for MAFR and BHFRIM were obtained from the Malaysian Meteorological Department.

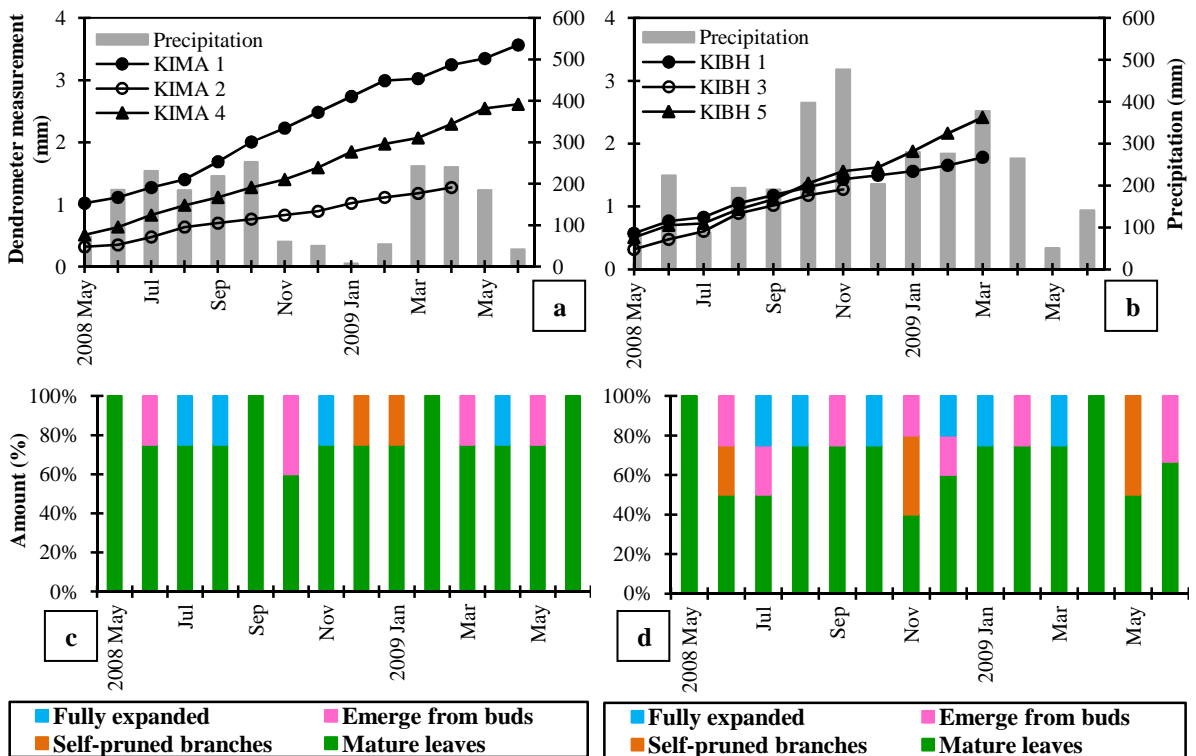


Fig. 1.4 Dendrometer measurement (**a and b**) and leaf phenology (**c and d**) of *Khaya ivorensis* grown in Mata Ayer Forest Reserve (MAFR, KIMA) and Bukit Hari FRIM (BHFRIM, KIBH). (**a and c**) KIMA. (**b and d**) KIBH. Precipitation data for MAFR and BHFRIM were obtained from the Malaysian Meteorological Department.

KIMA showed continuous radial growth until the last recordable month (Fig. 1.4a). Through phenological observation, KIMA trees were always covered with leaves (Fig. 1.4c). KIMA did not cease growing even when they shed some amount of their branches (self-pruning branches) in January and February 2009. KIBH showed continuous radial growth until the last recordable month (Fig. 1.4b). Through phenological observations, KIBH was always covered with leaves (Fig. 1.4d). Phenomena of self-pruned branches were also noticed, but not affecting radial growth (June and November 2008). Based on the similarity of radial growth, KIMA and KIBH were characterized as continuous growth species.

1.3.3 Growth rhythm of tropical rainforest trees

In this study, annual rhythm of radial growth in selected tropical rainforest trees grown in two different climates were characterized mostly based on meteorological conditions and leaf phenology. Alvim (1964) reported that *Tectona grandis* loses its leaves during the dry season in Western Java (periodically dry region), but behaves like an evergreen when cultivated in the wetter area of Eastern Java. Nishida *et al.* (2003) explained that the tree which received much water showed more active growth compared to those experienced drought. Nobuchi *et al.* (1995) clarified that *Hopea odorata* in Thailand formed more wood in the rainy season than in the dry season. Based on those examples, the author considered that similar scenario might be involved in tropical rainforest trees.

AEMA showed seasonal rhythm of radial growth. Active radial growth in AEMA was considered to have relation with much amount of precipitation, and all individuals were fully covered with leaves for photosynthesis. Slowing down of radial growth was considered to be affected by dry period, shedding all the leaves in January 2009. Ahmad Zuhaidi and Weinland (1995) reported a similar phenomenon of slow growth rate of *A. excelsa*, mentioning that *A. excelsa* had a pronounced deciduous habit with three months of leafless period. During this period, AEMA was expected to experience difficulties in water uptake. AEBH showed continuous radial growth without any obvious period of slowing down. In the meantime, AEBH experienced inconsistent leaf shedding. Both characteristics were considered to be the result that BHFRIM provides wet and humid environment.

HOMA showed seasonal rhythm of diameter growth. The active growths from May to October 2008 and March to June 2009 were considered to be the effect of sufficient water supply from the nearby small stream. Slowing down of radial growth in November 2008 to February 2009 was somehow arbitrary because leaves remained intact on the tree with a small portion of old leaves during the dry period. As mentioned, HOMA was planted nearby a water stream. Therefore, slowing down of radial growth in HOMA may be considered to be an effect of drying nearby stream during the dry period. Meanwhile, HOBH showed continuous radial growth rhythm.

KIMA and KIBH showed continuous radial growth during the measurement period. Although KIMA experienced dry period, there were no detectable slowing down of radial growth. The leaves remained intact on the tree, except that self-pruned branches were recorded concurrently. Lok and Ong (1999) mentioned that fast-growing *Khaya ivorensis* as a promising species for forest plantation possess the characteristics of good self-pruning. In this study, the exact mechanism of self-pruning in *K. ivorensis* was unknown. It was considered that during some period, lower branches did not receive enough sunlight to survive, or too dry to keep some leaves during the dry period.

1.3.4 *Characterization of cambial activity*

Plate 1.4 showed variation in anatomical features of cambium of AEMA and AEBH. As shown in Plate 1.4 (1d, 2b & 2c), similar problem occurred for all sections of *Hopea odorata* and *Khaya ivorensis*. The severely compressed sections were considered to be the effect of either fragile cambial region, minor damage on the cambial region during sampling, dull knife during sectioning or the tissue was incompletely infiltrated or embedded.

Table 1.1 summarizes the mean numbers of cambial zone (CZ) and enlarging zone (EZ) cells in rows with and without vessel element, and main anatomical features occurred in August 2008, November 2008, February 2009, and May 2009 for all species. In this section, diameter growth and leaf phenology from Fig 1.2 (a-d), Fig. 1.3 (a-d) and Fig. 1.4 (a-d) were referred to discuss on cambial activity.

Table 1.1 Mean numbers of cambial and enlarging zone cells and anatomical features of cambium

Sample	Month	Mean number of cells				Anatomical observation of cambium and enlarging zone features	
		Cambial zone		Enlarging zone		Pattern of division	Anatomical features
		Rows with vessel	Rows without vessel	Rows with vessel	Rows without vessel		
AEMA	Aug-08	9	12	14	20	AD	EVE
	Nov-08	6	8	10	14	AD	NFVE, EVE
	Feb-09	5	6	6	7	–	TRCW, TVE
	May-09	6	11	13	15	AD	EVE
AEBH	Aug-08	6	8	12	15	AD	EVE
	Nov-08	7	10	15	18	–	EVE
	Feb-09	6	9	13	16	–	EVE
	May-09	6	8	11	14	AD	EVE, TVE
HOMA	Aug-08	8	10	10	18	AD	EVE
	Nov-08	6	9	9	16	AD	EVE
	Feb-09	4	5	7	12	–	TRCW, EVE, TVE
	May-09	6	10	10	18	–	EVE
HOBH	Aug-08	5	7	6	9	–	EVE
	Nov-08	6	11	9	12	AD	NFVE, EVE
	Feb-09	5	9	7	10	–	EVE, TVE
	May-09	2	4	5	10	–	EVE, TVE
KIMA	Aug-08	4	8	18	22	–	EVE
	Nov-08	4	7	18	22	AD	EVE
	Feb-09	3	6	15	18	–	EVE
	May-09	4	9	11	18	AD	NFVE, EVE
KIBH	Aug-08	3	6	15	20	AD	EVE
	Nov-08	5	8	18	22	AD	NFVE, EVE
	Feb-09	4	8	17	18	AD	EVE
	May-09	2	5	10	12	–	EVE

Pattern of division: AD: Anticlinal division or just after division.

Anatomical features: NFVE: Newly-formed vessel element, EVE: enlarging vessel element, TVE: thickened vessel element, TRCW: Thick radial cell wall of flattened cells.

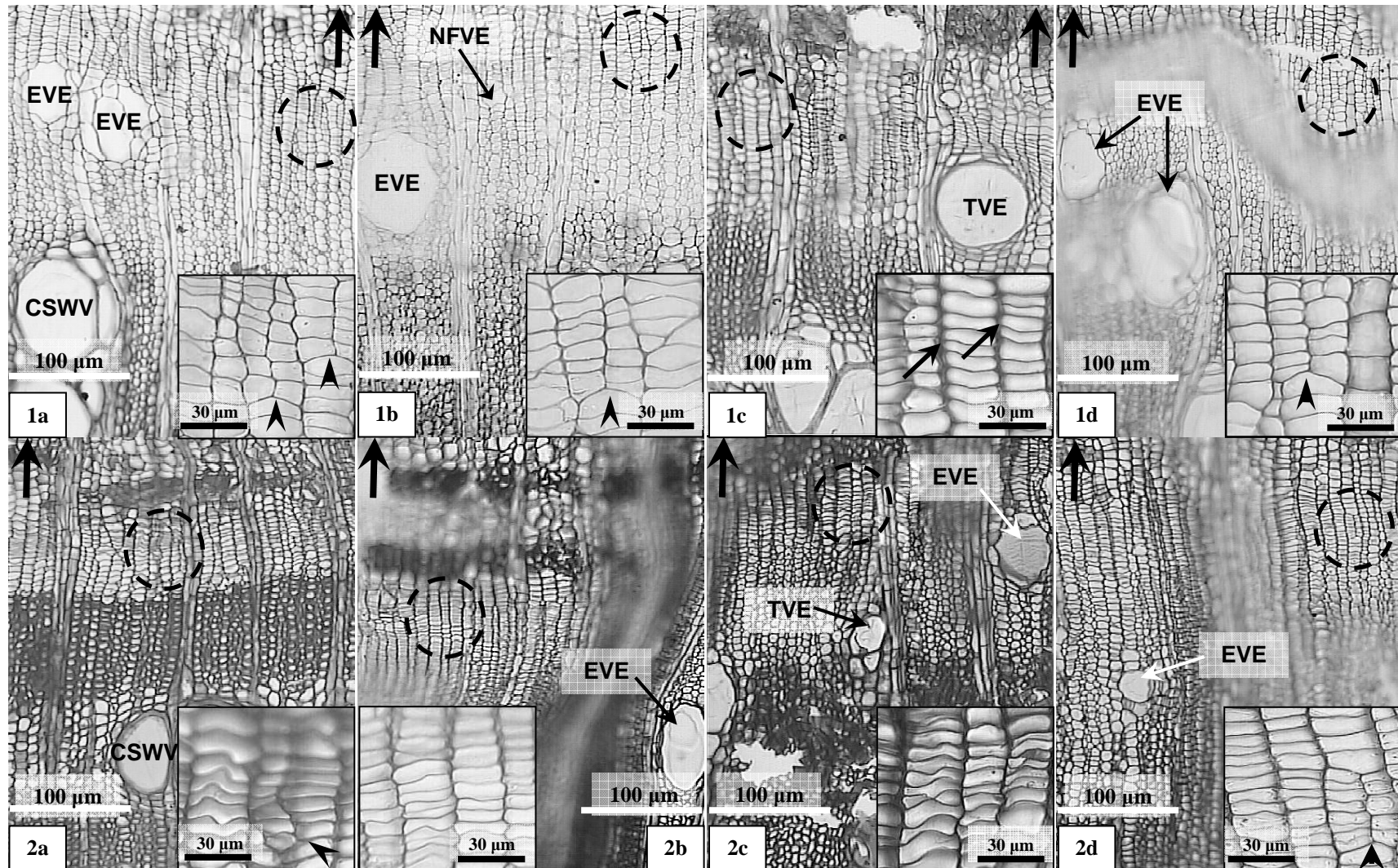


Plate 1.4 Transverse sections showing cambial zone and enlarging zone in *Azadirachta excelsa* growing in (1) Mata Ayer Forest Reserve and (2) Bukit Hari FRIM. (a) August 2008, (b) November 2008, (c) February 2009, and (d) May 2009. **NFVE**: newly formed vessel element, **EVE**: enlarging vessel element, **TVE**: Thickened vessel element, **CSWV**: completed secondary wall vessel. Inset images show the enlargement (from dashed circle) of anticlinal division or just after division (1a, 1b, 1d, and 2d), thick radial wall (1c), and no division (2a, 2b and 2c). Arrows indicate the direction of the bark side.

AEMA showed active cambial activity in August 2008 when it had a large number of cells in CZ and EZ with and without vessels (Table 1.1). During this period, AEMA grew in rainy period (Fig. 1.5a) and trees were covered with leaves (Fig. 1.2c). Based on Plate 1.4 (1a), the pattern of anticlinal division or just after the division (AD) and enlarged vessel element (EVE) were observed. AEMA showed less active cambium when cell numbers in both CZ and EZ with and without vessel decreased in November 2008 and February 2009, but not declined to zero. Although AEMA started to decrease the cell numbers in November 2008 (Fig. 1.5a), AD, EVE and newly-formed vessel element (NFVE) were observed (Plate 1.4, 1b). Although it was the start of a dry period, the trees continued to increase in diameter (Fig. 1.2a) and were still covered with leaves (Fig. 1.2c). Neither AD nor EVE was found in February 2009 (Plate 1.4, 1c), but the feature of TRCW in CZ was observed. During this period, trees just started to produce new shoots after experienced 3 to 4 months dry period (Fig. 1.2c). AEMA showed active cambial activity in May 2009 when the cell numbers in CZ and EZ with and without vessel increased, and the feature of AD and EVE were observed (Plate 1.4, 1d). Therefore, it was estimated that AEMA showed the tendency of seasonal cambial activity when the cambium was less active in November 2008 and February 2009 then active in May 2009, synchronized with the diameter growth and following the pattern of precipitation and changes in leaf phenology.

CZ with and without vessels in AEBH contained more than five cells and did not show conspicuous decrease, even if it experienced short period of less precipitation throughout the experimental period. However, the number of cells in EZ with and without vessel slightly decreased in February 2009 and May 2009 (Fig. 1.5b). AD was found only in August 2008 and May 2009, and EVE was observed in all sampled months (Table 1.1). In February 2009, EVE and TVE were observed (Plate 1.4, 2c) while AD and EVE were found in May 2009 (Plate 1.4, 2d). Compared to AEMA, AEBH did not showed any TRCW, except for TVE was observed in February 2009. Based on Fig. 1.5b, AEBH did not show any typical seasonal cambial activity in CZ with and without vessel, but showed seasonal rhythm in EZ with and without vessel.

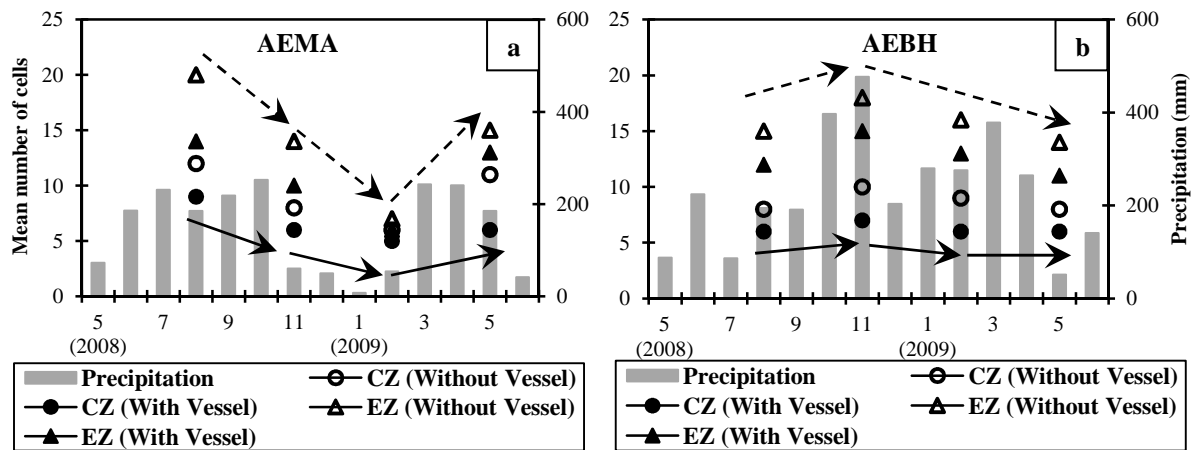


Fig. 1.5 Relationship of cambial zone (CZ) and enlarging zone (EZ) cells of *Azadirachta excelsa* grown in (a) Mata Ayer Forest Reserve and (b) Bukit Hari FRIM with amount of precipitation from May 2008 to Jun 2009. Number of cells was counted from transverse sections of August 2008, November 2008, February 2009, and May 2009. Arrows indicate the estimated tendency of activity in cambial zone and dashed arrows indicate the estimated tendency of activity in enlarging zone.

HOMA showed active cambial activity in August 2008 when it had high cell numbers in CZ and EZ with and without vessel (Fig. 1.6a). AD and EVE were observed in this sampled month (Table 1.1). Cell numbers in both CZ and EZ with and without vessel decreased continuously in November 2008 and February 2009, and did not decline to zero. In CZ without vessels, there was no AD, but TRCW feature was observed in February 2009. Features of EVE were observed in EZ with vessel during this two periods, as well as TVE in February 2009. During less active cambium, HOMA experienced dry period and had some portion of shed leaves (Fig. 1.3c). In the meantime, HOMA also developed new shoots during dry period. HOMA again showed active cambial activity when the number of cells in CZ and EZ with and without vessel increased in May 2009. Therefore, it was estimated that HOMA showed the tendency of seasonal cambial activity when the cambium was less active in November 2008 and February 2009 then active in May 2009. These observations synchronized with diameter growth measured by dendrometer (Fig. 1.3a), following the pattern of precipitation.

HOBH showed active cambial activity throughout the experimental period when considered from cells number in CZ and EZ, and the appearance of EVE feature (Table 1.1). Little difference between EZ with and without vessel (Fig.1.6b) was affected only by the occurrence of EVE and TVE. Cell numbers in EZ without vessel in May 2009 remained the same as those in November 2008, probably focused on the increase in girth with either fiber or parenchyma cells. Meanwhile, cell

numbers in EZ with vessel decreased because some portion of xylem tissue was occupied with EVE and TVE. This may depend on the size of vessel element and it may take over some number of radial cell files; for example four to five radial cell files for one vessel element. Therefore, it was estimated that HOBH showed active cambial activity and it was synchronized with continuous radial growth measured by dendrometer (Fig. 1.3b).

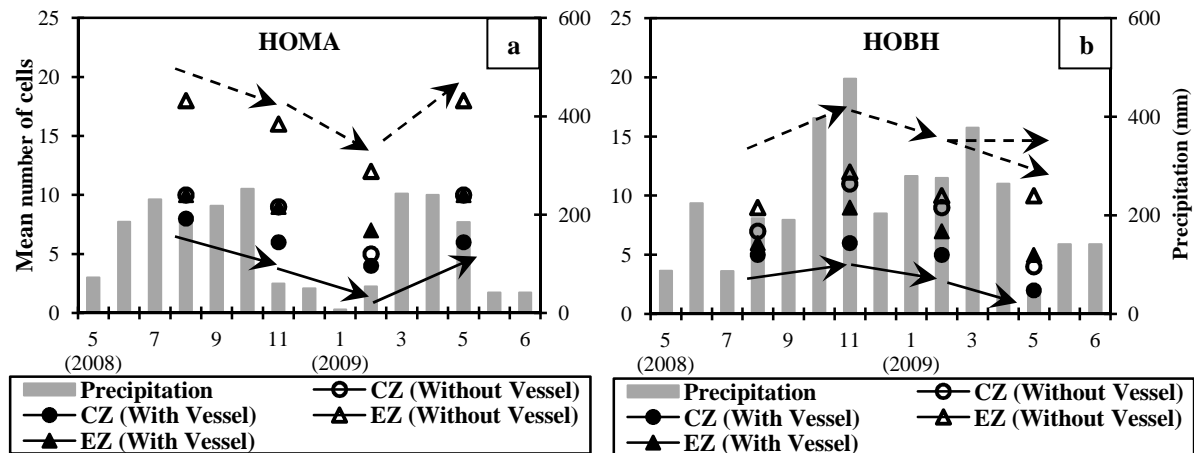


Fig. 1.6 Relationship of cambial zone (CZ) and enlarging zone (EZ) cells of *Hopea odorata* grown in (a) Mata Ayer Forest Reserve and (b) Bukit Hari FRIM with amount of precipitation from May 2008 to Jun 2009. Number of cells was counted from transverse sections of August 2008, November 2008, February 2009, and May 2009. Arrows indicate the estimated tendency of activity in cambial zone and dashed arrows indicate the estimated tendency of activity in enlarging zone.

KIMA showed active cambial activity along the experimental period. Number of cells in CZ was always less than 10 but up to 22 cells in EZ for each month (Table 1.1). KIMA showed slightly decreased CZ and EZ cell numbers from November 2008, but the features of AD and EVE were observed. Cell numbers in CZ increased again in May 2009 when the tree grew in rainy period and features of AD and EVE were again observed, as well as NFVE in May 2009. However, cell numbers in EZ with vessels continued to decrease while EZ without vessel remained the same as in February 2009 (Fig. 1.7a). The decrease of cell numbers in EZ were not interpreted as less active cambium since there were EVE observed in EZ. Therefore, it was estimated that KIMA showed the tendency of continuous active cambium with no effect of dry period, and trees were always covered with leaves (Fig 1.4c).

KIBH showed active cambial activity for each month, noticed by occurrence of AD and EVE features in most of the months, even the number of cells in CZ and EZ decreased from November 2008 (Table 1.1). Most active cambial activity happened in November 2008 when the number of CZ

and EZ cells increased and the features of AD and NFVE were found. During this period, KIBH received high amount of precipitation. Based on Fig. 1.7b, it was estimated that KIBH were always active in cambial activity since they always received much precipitation and trees were always covered with leaves (Fig. 1.4d).

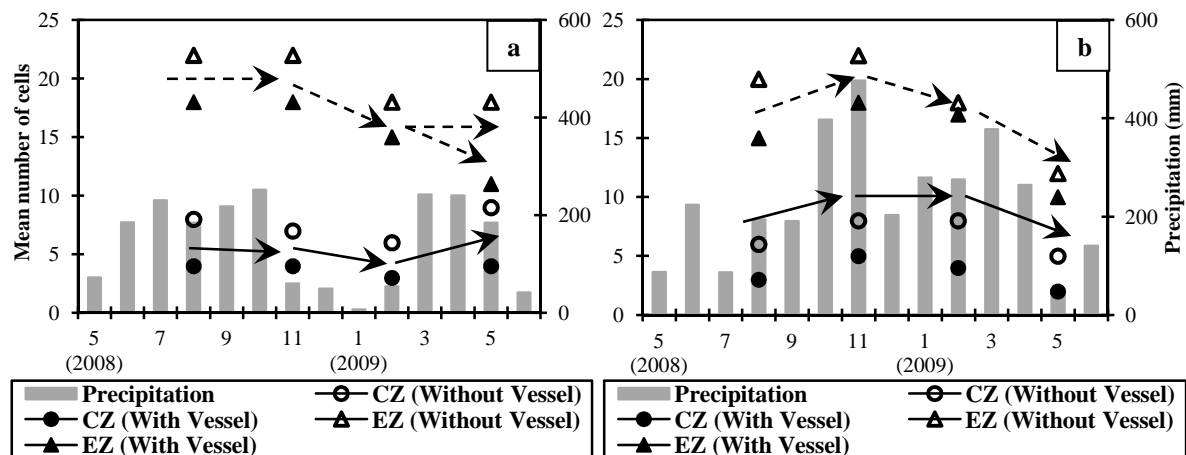


Fig. 1.7 Relationship of cambial zone (CZ) and enlarging zone (EZ) cells of *Khaya ivorensis* grown in (a) Mata Ayer Forest Reserve and (b) Bukit Hari FRIM with amount of precipitation from May 2008 to Jun 2009. Number of cells was counted from transverse sections of August 2008, November 2008, February 2009, and May 2009. Arrows indicate the estimated tendency of activity in cambial zone and dashed arrows indicate the estimated tendency of activity in enlarging zone.

1.3.5 Periodicity of cambial activity

Periodicity in cambial activity produces growth rings in the stems of perennial plants. According to Larson (1962), ring formation in temperate trees is related to auxin gradients. In spring, the first flush of leaf is rapid and the resulting high auxin synthesis stimulates production of a zone of earlywood cells throughout the cambial region of a tree. As the season advances terminal growth ceases, auxin synthesis declines, and the latewood formation is initiated. As a rule, the period of higher cambial activity coincides with the period of higher photosynthesis production (Kozlowski, 1962).

Active cambial activity in all studied species were judged from high and increased number of CZ and EZ cells, and occurrence of AD, NFVE, and EVE.. According to Kozlowski (1962), diameter growth seems to depend primarily on current photosynthesis; it is possible that higher cambial activity during an active growing period results from favorable conditions for photosynthesis associated with limited flushing at that time. AD was considered to be triggered by hormonal activity and supplied

photosynthates from active leaves. Rao and Kisbore (1999) reported that trees that have access to water throughout a year would show continuous cambial activities. The feature of NFVE and EVE were considered to be affected by much amount of available water in soil.

Less active cambium in AEMA and HOMA were considered based on low and decreased cell numbers in CZ and EZ. In some occasion, TRCW can be found in CZ and TVE in EZ. Nobuchi and Hori (1998) reported that the thicker radial walls of cells (TRCW) in cambial zone are one of the features happened in temperate trees during dormant season. However, occurrence of TRCW during the dry and leaf shedding period was considered as one of the peculiar features of tropical trees which indicate short or non dormancy in cambial activities. This is because the number of CZ cells did not reach zero and radial growth continued in a slow pace.

Compared to other species, KIMA and KIBH had more cells in EZ than in CZ. It was considered that the fast-growing *K. ivorensis* focused to enlarge faster while cell division occurred at the same time. It should be subject of future research in investigating the characteristics of cambial activity in tropical fast-growing species.

1.3.6 *Cambial response on cambial marking*

Transverse section of the wound tissue induced by knife marking was shown in Plate 1.5. The wound tissue formed was divided into two zones although it showed a very complicated structure. In zone 1, the cambial initials and cells in differentiating zone had been directly affected by knife marking. They had been crushed and cell wall formation in the living cells had been interrupted during knife insertion. Therefore, cells in this zone retained the cell wall organization at the time of marking even if they were deformed. Under polarized light, the birefringence of S₁ layer can be observed in the bark-side outermost cells (Plate 1.6a). Under bright light, the residue of deformed cells was attached on the cells (Plate 1.6b). It was considered that the cells pointed by arrowheads were those initiated the S₁ layer formation at the time of marking.

Anatomical features of indirectly affected zone (zone 2) showed a region of normal xylem tissue, radially flattened cells, and small diameter vessels. The callus-like cells in zone 1 were considered to have been formed by ray parenchyma cells to fill the gap formed after knife insertion.

This layer included the residues of cambial and enlarging zones at the time of marking. The callus-like cells were considered to correspond to the location of enlarging zone at the time of marking. Therefore, it was referred to deduce the feature or showed a pattern of cambial zone.

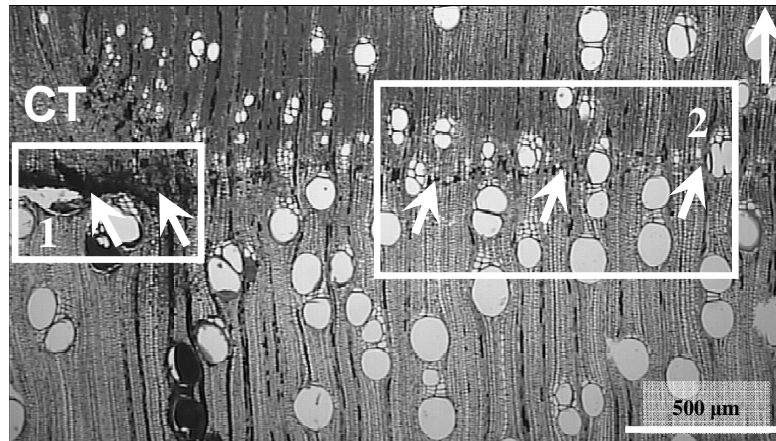


Plate 1.5 Transverse section of *Azadirachta excelsa* showing wound tissue with two marked zones. **Zone 1:** tissue directly affected by marking. **Zone 2:** tissue indirectly affected by knife marking. Arrows in Zone 1 and Zone 2 indicate the deduced position of cambial cells at the time of marking. CT: callus tissue. Arrow indicates the direction of bark side.

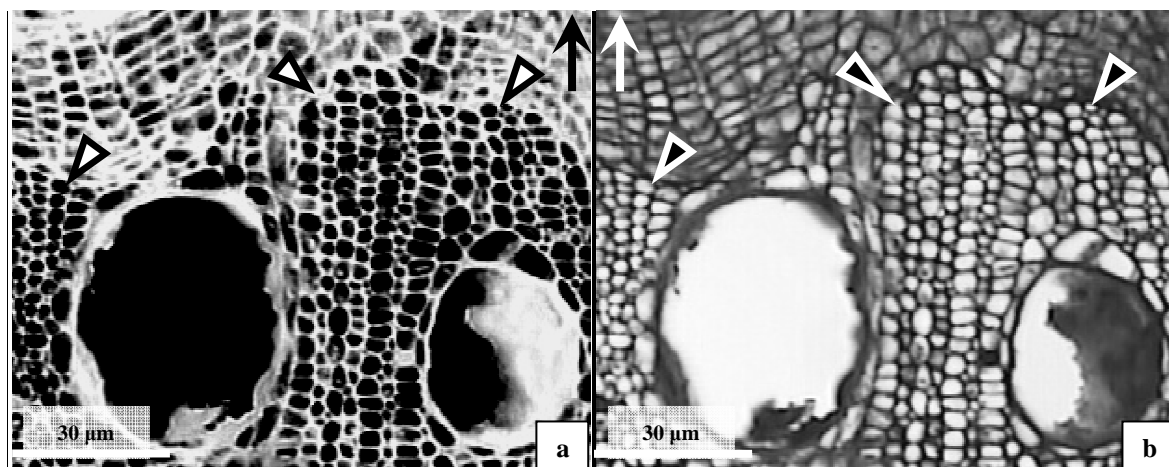


Plate 1.6 Transverse sections correspond to zone 1 observed under (a) polarized microscope and (b) conventional microscope in *A. excelsa*. Arrowheads indicate the deduced site of the initiation of S_1 layer formation. Arrows indicate the direction of bark side.

After marking, region with small vessels were observed (Plate 1.7). It was considered that the small vessel diameter was an effect of abnormal differentiation from the cambial cells, by being affected by knife insertion. Radially flattened cells were also included in those regions with small vessels. It was considered that the radially flattened cells remained undifferentiated after marking and retained their structural characteristics, and is similar to those in the cambial zone.

The arrowhead in Plate 1.7b was a point where number of cell rows increased tangentially. The increased number of cell rows was considered to have caused by the anticlinal division of cambial cells. The line which connects the innermost points of anticlinal divisions was considered theoretically to be the location of cambium at the time of knife insertion. This line coincided with the line connecting the locations of the innermost small-size vessels. Therefore, this line was adopted as the marker of cambial zone at the time of marking. The anatomical features of wound tissue and deduced cambial zone in *Hopea odorata* and *Khaya ivorensis* were basically the same as in *A. excelsa*.

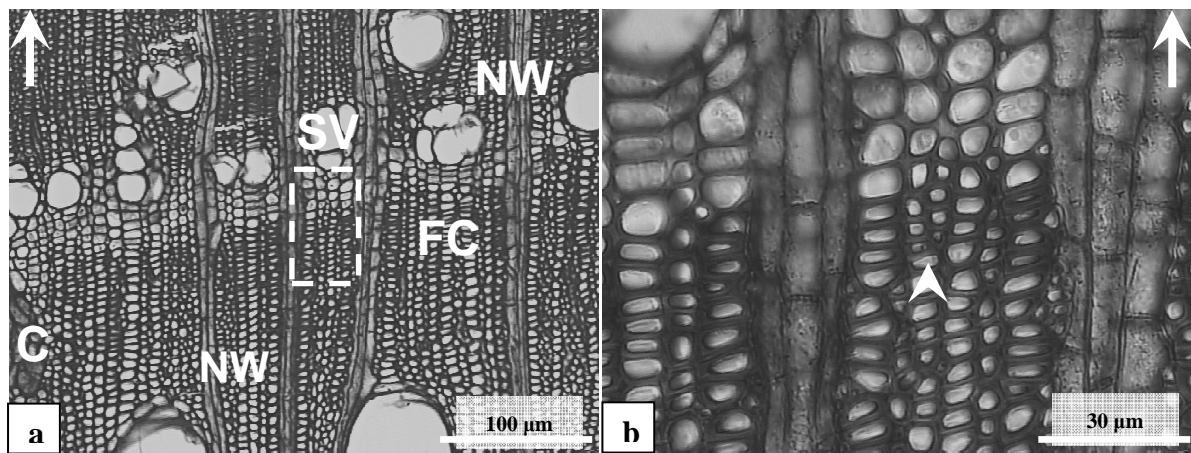


Plate 1.7 Transverse sections of *A. excelsa* showing indirectly affected zone, corresponding to Zone 1 in Plate 1.5. **(a)** Anatomical features appeared in indirect affected zone. **NW**: normal wood formed before marking, **CC**: callus-like cells corresponding to enlarging zone at the time of marking as deduced in indirect affected zone, **FC**: radially flattened cells, **SV**: the layer included with small vessels formed after marking. **(b)** Enlargement of dashed box in **(a)**, featuring increased of cell rows (arrowhead). Arrows indicate the direction of bark side.

Fujiwara (1992) estimated the position of cambial and enlarging zone at the time of marking in the directly affected zone. However, the cells of directly affected zone were generally crushed by knife insertion. Therefore, it was difficult to deduce the exact position of cambium at the time of marking. Nobuchi et al. (1995) estimated that the cambial zone was located in the indirectly affected zone by the knife insertion.

There were some reports on the formation of traumatic resin canals after cambial marking (e.g. Kuroda and Shimaji 1983, Shiokura 1989, Nobuchi et al. 1995). In this study, the feature of traumatic resin canals occurred after knife insertion (Plate 1.8a & b). They were formed longitudinally and tangentially. Generally, they were formed by disintegration of axial or ray parenchyma cells due to the injury (Kuroda and Shimaji 1983). The wall of epithelial cells of traumatic resin canals was thicker

than that of the normal resin canal. In this research, all species studied formed the traumatic resin canals after pinning injury.

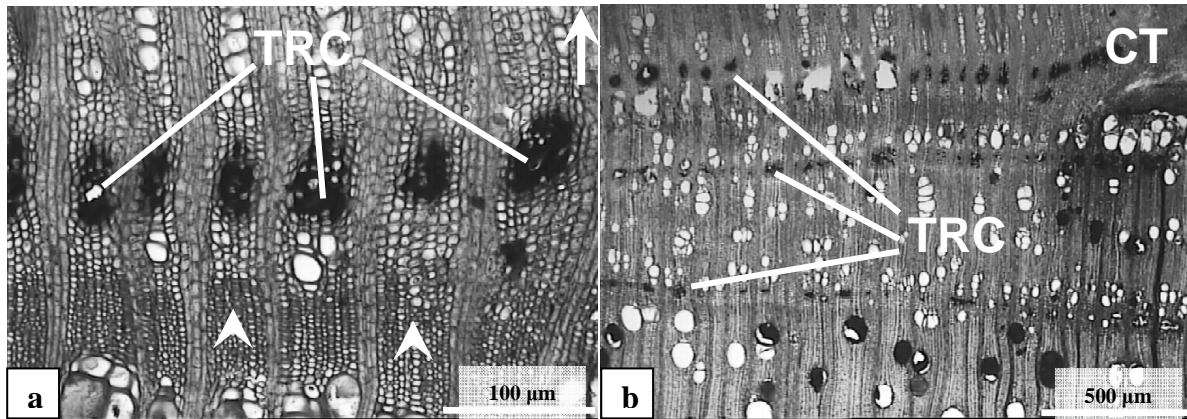


Plate 1.8 Transverse sections of *A. excelsa* showing traumatic resin canals (TRC) located on the bark side of the deduced site of cambial cells (arrowheads). **(a)** Band of traumatic resin canal formed after knife insertion. **(b)** Overlapping of traumatic resin canal bands caused by marking in different period. **CT**: Callus tissue. Arrow indicates the direction of bark side.

The traumatic resin canals were located towards the bark side of the estimated line of cambial cells at the time of marking. In some occasions, the formation of this feature can be overlapping (Plate 1.8b). Kuroda and Shimaji (1983) reported that the estimated position of cambial zone at the time of marking was located towards the bark side of epithelial cells constituting resin canals. Shiokura (1989) also used gum/resin canals as the position of cambium during marking. Nobuchi et al. (1995) stressed that traumatic resin canals cannot be used as the marker of cambial zone because they did not reveal the exact position of cambium at the time of marking. In this study, therefore, traumatic resin canals were not used as the cambial marker at the time of marking since they were considered to be formed by traumatic events, such as mechanical injury with knife. Appearance of overlapped traumatic resin canal also causes difficulties in investigating the anatomical changes along the radial direction from marked cambium, such as the appearance of ring-like structure. It was considered that modification of cambial marking technique in tropical rainforest trees must be prioritized to enhance the study of wood formation.

1.4 Summary

The application of dendrometer, cambial observation and cambial marking coupled with phenological observation and anatomical investigation in the studied species showed potential to investigate wood formation in tropical rainforest trees in Peninsular Malaysia.

The studied species had ring-like structures, featured by band of axial parenchyma and zone with thick-walled fibers. The occurrence of these features stimulated the interest for further investigation of growth ring occurrence in tropical rainforest trees. Some of the studied species had seasonal growth rhythm when radial growth slows down during a dry period and shedding of leaves. This informed that radial growth in some tropical rainforest trees reacted to changes in precipitation and leaf phenology. Seasonal cambial activity were clarified when the cell numbers in the cambial and enlarging zones decreased, and no newly-formed or enlarged vessel elements were observed during a dry period or that of low precipitation and shedding of leaves. Through cambial marking, it was shown that cambium of tropical rainforest trees react strongly to mechanical injury (e.g. knife marking).

Seasonal rhythm of radial growth, seasonal cambial activity and cambial response to mechanical injury in studied species were investigated. However, further attention is required to enhance the study of wood formation in tropical rainforest trees:

1. Investigate the occurrence of growth rings in more individuals of selected tropical rainforest tree species and determine their anatomical features.
2. Shorten the duration of dendrometer measurement and phenological observation to elucidate clearer pattern of radial growth and leaf phenology.
3. Substitute the anatomical observation of cambium with other cambial monitoring method, to reduce the damage on tree stem and obtain more data to elucidate clear rhythm of cambial activity in tropical rainforest trees.
4. Minimize the wound caused by cambial marking for precise observation of anatomical changes during a known period.
5. Apply isotopic measurement to check the relation between anatomical changes in xylem and water availability.

Chapter 2

Occurrence and anatomical features of growth rings in tropical rainforest trees in Peninsular Malaysia

2.1 Introduction

There were some basic studies reporting growth rings in various tropical species and their potential for tropical dendrochronology (e.g., Roig et al. 2005, Maingi 2006, Worbes and Fichtler 2011). Thus, investigating the characteristics of growth rings in tropical trees and identifying the species that have potential for dendrochronological studies in the tropics represent a major new endeavor in tropical dendrochronology. Stale (1999) has proposed to identify the temperate or subtropical species that have known dendrochronological value and a wide natural distribution and to extend the survey into the tropics. However, the distribution of these species is limited to certain growing conditions and they are not widely found in tropical rainforests.

In this chapter, the author examined growth rings in common tropical rainforest tree species in Peninsular Malaysia. The author described and categorized the anatomical features of growth ring markers in trees from two plantations and a natural forest. The study sites and studied species discussed in Chapter 3, Chapter 4 and Chapter 5 were the same as in this chapter.

2.2 Materials and Methods

2.2.1 *Study site*

The study trees were selected from two plantations and one natural forest (Fig. 1). Both forest plantations are managed by the Forest Research Institute Malaysia, and are located at Mata Ayer Forest Reserve, Perlis (MAFR, 6°40'N, 100°15'E) and Bukit Hari, Forest Research Institute Malaysia, Selangor (BHFRIM, 3°14'N, 101°38'E). The climate of MAFR is classified as tropical monsoon (A_m according to Köppen-Geiger climate classification, Peel et al. 2007) with a 2–3-month-long dry season from December to February. The climate of BHFRIM is classified as tropical rainforest (A_f according to Köppen-Geiger climate classification), in which the monthly precipitation is usually much greater than 100 mm. The natural forest is a secondary disturbed lowland dipterocarp forest,

Ayer Hitam Forest Reserve, Selangor (AHFR, 3°1'N, 101°39'E). The climate of AHFR is also classified as tropical rainforest, but with a short dry period for 1–2 months in June and July.

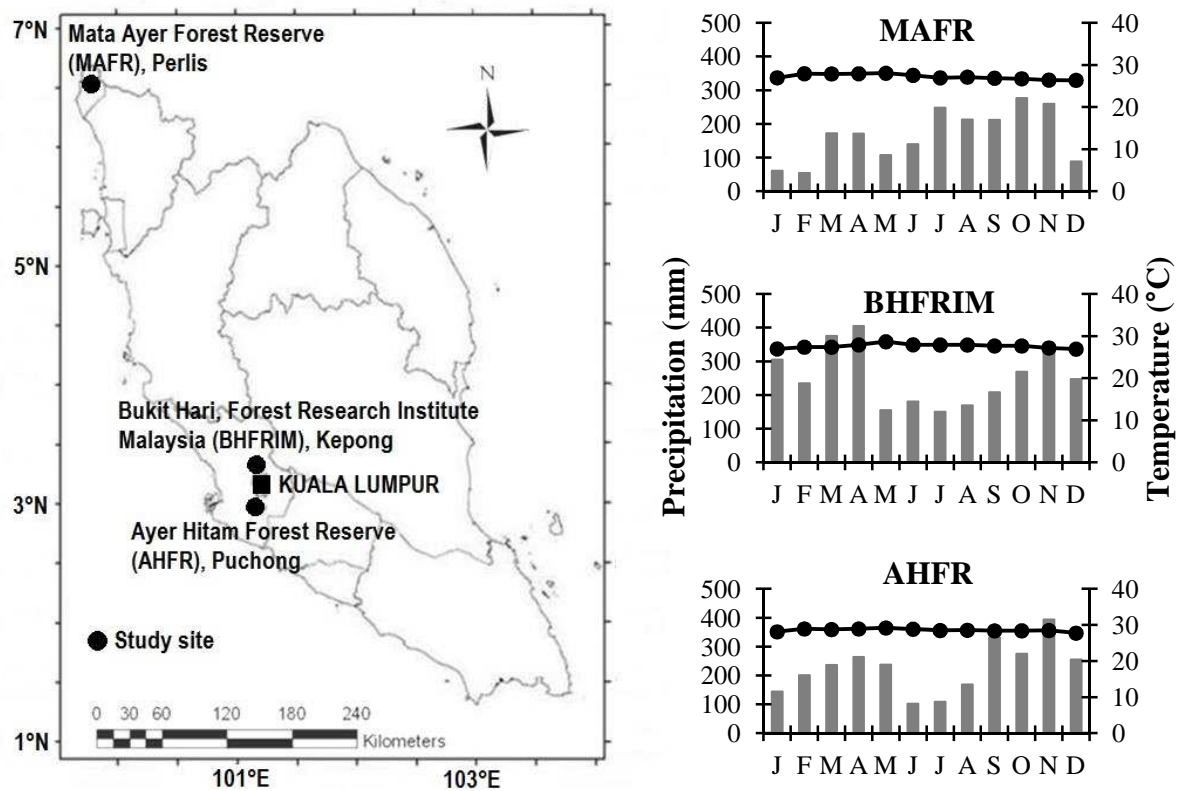


Fig. 2.1 Location of research sites and climate diagram for each area. Monthly precipitation (bar) and temperature (line) are the mean from 2000 to 2011. Climate data for the Ayer Hitam Forest Reserve (AHFR) was provided by Universiti Putra Malaysia, and data for the Mata Ayer Forest Reserve (MAFR) and Bukit Hari, Forest Research Institute Malaysia (BHFRIM) were obtained from the Malaysian Meteorological Department.

2.2.2 Study species and methods

Table 2.1 summarizes the selection of tree species, as well as the information regarding their growth rings from the literature available (FRIM 1993, PROSEA 1994a, b, c, Ogata et al. 2008). Occurrence and anatomical features of growth rings were investigated in five tree species, belonging to three families, planted in MAFR, five tree species, belonging to four families, planted in BHFRIM, and 26 tree species, belonging to 18 families that grow naturally in AHFR. All species selected in MAFR were planted in 2000 and trees in BHFRIM were planted in 1998 (*Azadirachta excelsa*, *Dyera costulata* and *Hopea odorata*), 1999 (*Shorea roxburghii*) and 2000 (*Peronema canescens*). Selected tree species in AHFR are common species that grow in primary and secondary forests.

The investigation of anatomical features was conducted using wood cores, as destructive methods are not permitted. Depending on the number and size of trees available in the study sites, up to 20 individuals were sampled for each species with an increment borer (diameter = 5.15 mm). Wood cores were extracted from the north, west, east, and south sides of each tree stem at breast height at the end of December 2011. Each core was examined macroscopically under an Olympus SZX12 stereomicroscope. Transverse sections 20–30 μm in thickness were cut using a sliding microtome and double-stained with safranin and fast green. All transverse sections were observed microscopically under an Olympus BX50 compound microscope.

2.2.3 *Description of anatomical feature of growth rings*

The author classified growth rings as (A) well defined, (B) poorly defined, and (C) absent, on the basis of microscopic observation. Following Worbes (1985), the IAWA Committee (1989), and Carlquist (2001), the author then considered the anatomical features of growth ring boundaries for each species and classified them into one or combination of the following five types:

1. Fiber zones (FZ): The tissue is mainly composed of wood fiber, which appears as a tangentially darker band macroscopically. Microscopically, it is a zone with fewer or no vessels and/or axial parenchyma cells.
2. Radially flattened fibers (RF): The sample contains radially narrow fibers.
3. Marginal parenchyma (MP): There is a tangentially arranged axial parenchyma band at the initial or terminal stage of xylem formation.
4. Thick-walled fibers (TF): The cell wall changes from thin-walled cells at the beginning of the growth period to thick-walled cells at the end of the growth period.
5. Variation in the vessel sizes and/or density (VSD): The vessel diameter and/or density changes from large diameter and low density to small diameter and high density in the xylem layer.

Some samples presented difficulties and their growth ring could not be easily classified. The difficulties encountered were categorized as:

1. Intermittent growth rings (IT): non-periodical and sporadic occurrence of growth rings.
2. Ambiguous growth rings (AR): indefinite or indiscernible ring boundary.

3. Discontinuous growth rings (DC): discontinuous along a circumference.
4. Occurrence of resin canals and/or traumatic canals (RTC): resin canals and/or traumatic canals were included in parenchyma band.

2.3 Results

2.3.1 Anatomical features of growth rings

Table 1 summarizes the degree of growth ring distinctiveness, types of growth rings, and types of difficulties encountered in each species studied. Unlike FRIM (1993) and PROSEA (1994a, b, c), the present study revealed that even though some species investigated formed growth rings, these were either weak or faint. All five types of growth rings were observed and some species showed a combination of several types.

Growth rings were well defined in *Peronema canescens* (Verbenaceae, MAFR and BHFRIM), featuring ring-porous porosity (Plate 2.1a & 2.1b), and *Intsia palembanica* (Leguminosae, AHFR), featuring marginal parenchyma (Plate 2.5a & 2.5b). In these species, the rings showed essentially the same pattern and their occurrence might be caused by environmental factors. Growth rings were absent in 10 species. Poorly defined growth rings were encountered in 17 species.

Fiber zones (FZ) were most frequently observed as growth ring boundaries in eight species. These species were *Garcinia urophylla*, *Pternandra echinata*, *Syzygium polyanthum*, *Ochanostachys amentacea*, *Paropsia vareciformis* (Plate 2.2), *Aidia densiflora*, *Diplospora malaccense*, and *Gonystlus confusus*. All these species grow naturally in the tropical rainforest climate of AHFR.

Growth rings featuring radially flattened fibers (RF) were identified in five species, on the basis of microscopic observation. These species were *Dyera costulata* (BHFRIM and AHFR), *G. urophylla* (AHFR), *Macaranga gigantea* (AHFR), *Litsea costalis* (AHFR, Plate 2.3), and *A. densiflora* (AHFR).

Like RF growth rings, those that featured thick-walled fibers (TF) and clear ring boundaries were observed microscopically. Fiber wall thickness changed from thin-walled at the beginning of

one growth period to thick-walled at the end of the period. Tree species featuring TF as growth rings were *G. urophylla* (Plate 2.4) and *Shorea parvifolia*, both of which grow naturally in AHFR.

Five species showed marginal parenchyma (MP). This feature was distinct macroscopically, depending on the spacing between boundaries (Plate 2.5a). Microscopically, MP appears as parenchyma bands associated with abrupt changes in radial diameter or wall thickness of fibers (Plate 2.5b). Tree species classified as MP were *Azadirachta excelsa* (MAFR, AHFR), *I. palembanica* (AHFR), *Sindora wallichii* (AHFR), *Timonius wallichianus* (AHFR), and *Gironniera nervosa* (AHFR).

Clear variation in the vessel sizes and/or density (VSD) was found only in *P. canescens* (MAFR and BHFRIM), although weak VSD types included *A. excelsa* (BHFRIM and AHFR) and *Gmelina arborea* (MAFR). We classified both *A. excelsa* and *G. arborea* as weak VSD because the changes in vessel sizes were not as clear as in *P. canescens* and there were only faint differences in vessel density. In this study, the author primarily observed the anatomical features and did not measure the size of vessels, but the author were able to determine the position of growth ring boundaries as the vessel feature changed from high density and small diameter to low density and large diameter in one xylem layer (e.g., Plate 2.6). *A. densiflora* and *G. urophylla* growing in AHFR showed a combination of types of fibers in their growth rings, and *A. excelsa* (AHFR) showed a combination of growth rings between the MP and VSD types within the xylem layer.

Table 2.1 Selection of tree species and the characteristics of growth ring in the literature and the present study.

Study site / Family / Species	Characteristics of growth ring			
	Literature	Present study		
		Distinctiveness	Type	Difficulties
Mata Ayer Forest Reserve				
Dipterocarpaceae				
<i>Hopea odorata</i>	⁽¹⁾ Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC
<i>Shorea roxburghii</i>	⁽²⁾ Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC
Meliaceae				
<i>Azadirachta excelsa</i>	⁽³⁾ Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	MP	IT, AR, RTC
Verbenaceae				
<i>Gmelina arborea</i>	Marked by differences in pore size and fiber wall thickness (PROSEA 1994a)	B	VSD	AR
<i>Peronema canescens</i>	⁽⁴⁾ Marked by differences in vessel frequency and size (ring-porous porosity) (PROSEA 1994a, Ogata et al. 2008)	A	VSD	–
Bukit Hari, FRIM, Selangor				
Apocynaceae				
<i>Dyera costulata</i>	⁽⁵⁾ 1. Marked by periodic layers without wood parenchyma (FRIM 1993) 2. Marked by differences in fiber wall thickness (PROSEA 1994b)	B	RF	AR
Dipterocarpaceae				
<i>Hopea odorata</i>	⁽¹⁾ Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC
<i>Shorea roxburghii</i>	⁽²⁾ Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC
Meliaceae				
<i>Azadirachta excelsa</i>	⁽³⁾ Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	VSD	AR, RTC
Verbenaceae				
<i>Peronema canescens</i>	⁽⁴⁾ Marked by differences in vessel frequency and size (ring-porous porosity) (PROSEA 1994a, Ogata et al. 2008)	A	VSD	–
Ayer Hitam Forest Reserve				
Apocynaceae				
<i>Dyera costulata</i>	⁽⁵⁾ 1. Marked by periodic layers without wood parenchyma (FRIM 1993) 2. Marked by differences in fiber wall thickness (PROSEA 1994b)	B	RF	AR
Clusiaceae				
<i>Garcinia urophylla</i>	If present, marked by different regions of fewer parenchyma bands (PROSEA 1994c)	B	FZ, TF, RF	AR
Dipterocarpaceae				
<i>Hopea odorata</i>	⁽¹⁾ Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC
<i>Shorea acuminata</i>	Indistinct (FRIM 1993, PROSEA 1994a)	C	–	RTC

Table 2.1 (continued).

Study site / Family / Species	Characteristics of growth rings			
	Literature	Present study		
		Distinctiveness	Type	Difficulties
Ayer Hitam Forest Reserve				
Dipterocarpaceae				
<i>Shorea parvifolia</i>	Indistinct (FRIM 1993, PROSEA 1994a)	B	TF	AR, RTC
Euphorbiaceae				
<i>Macaranga gigantea</i>	Indistinct (PROSEA 1994c)	B	RF	AR
Lauraceae				
<i>Litsea costalis</i>	1. Concentric markings produced by terminal bands or wood parenchyma (FRIM 1993) 2. Differences in fiber wall thickness and radial fiber diameter (PROSEA 1994b)	B	RF	IT
Leguminosae				
<i>Intsia palembanica</i>	1. Produced by terminal layers of wood parenchyma (FRIM 1993) 2. Marked by marginal parenchyma bands and/or differences in vessel frequency (PROSEA 1994a)	A	MP	–
<i>Sindora wallichii</i>	1. Produced by terminal layers of wood parenchyma (FRIM 1993) 2. Marked by terminal layers of wood parenchyma (PROSEA 1994a)	B	MP	IT, RTC
Melastomataceae				
<i>Pternandra echinata</i>	Indistinct (PROSEA 1994c)	B	FZ	AR
Meliaceae				
<i>Aglaia oligophylla</i>	Indistinct (PROSEA 1994b)	C	–	–
<i>Azadirachta excelsa</i>	⁽³⁾ Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	VSD, MP	AR, DC, RTC
Moraceae				
<i>Artocarpus scortechinii</i>	1. Indistinct (FRIM 1993) 2. Usually indistinct, but when present generally marked by long wings of parenchyma and smaller pores (PROSEA 1994b)	C	–	–
<i>Streblus elongatus</i>	Indistinct, occasionally visible, indicated by darker colored bands with relatively few vessels and parenchyma (PROSEA 1994c)	C	–	–
Myrtaceae				
<i>Syzygium polyanthum</i>	Indistinct or inconspicuous, but occasionally concentric with 3-5 mm-thick bands with few or no vessels (PROSEA 1994b)	B	FZ	AR
Olacaceae				
<i>Ochanostachys amentacea</i>	Indistinct (PROSEA 1994b)	B	FZ	AR
Passifloraceae				
<i>Paropsia vareciformis</i>	No literature	B	FZ	AR

Table 2.1 (continued).

Study site / Family / Species	Characteristics of growth rings			
	Literature	Present study		
		Distinctiveness	Type	Difficulties
Ayer Hitam Forest Reserve				
Rhizophoraceae				
<i>Gynotroches axillaris</i>	Indistinct (PROSEA 1994c)	C	–	–
<i>Pellacalyx axillaris</i>	Indistinct (PROSEA 1994c)	C	–	–
Rubiaceae				
<i>Aidia densiflora</i>	No literature	B	RF, FZ	IT, AR
<i>Diplospora malaccense</i>	No literature	B	FZ	IT, AR
<i>Timonius wallichianus</i>	Sometimes visible, indicated by layers of marginal parenchyma (PROSEA 1994c)	B	MP	IT, AR, DC
Sapindaceae				
<i>Pometia pinnata</i>	1. Vague concentric marking produced by terminal layers of wood parenchyma may be visible on end surface (FRIM 1993) 2. Marked by concentric bands of marginal parenchyma (PROSEA 1994a)	C	–	–
Sapotaceae				
<i>Palaquium maingayi</i>	1. Generally absent but occasionally concentric layers of dense fibers may occur that simulate growth rings (FRIM 1993) 2. Indistinct, if present, marked by differences in spacing on tangential parenchyma bands and/or in fiber wall thickness on either side of the ring boundary (PROSEA 1994a)	C	–	–
Thymelaeaceae				
<i>Gonystlus confusus</i>	Indistinct (FRIM 1993, PROSEA 1994a)	B	FZ	AR
Ulmaceae				
<i>Gironniera nervosa</i>	Indicated by banded parenchyma (PROSEA 1994c)	B	MP	IT, DC

⁽¹⁾ to ⁽⁵⁾ Same tree species and growth ring information based on the literature.

Distinctiveness: (A) Well defined, (B) Poorly defined (with difficulties), (C) Absent.

Type: (FZ) Fiber zones, (RF) Radially flattened fibers, (MP) Marginal parenchyma, (TF) Thick-walled fibers, (VSD) Variations in vessel size and/or density.

Difficulties: (IT) Intermittent growth ring, (AR) Ambiguous growth ring, (DC) Discontinuous growth ring, (RTC) Occurrence of resin and/or traumatic canal in parenchyma band.

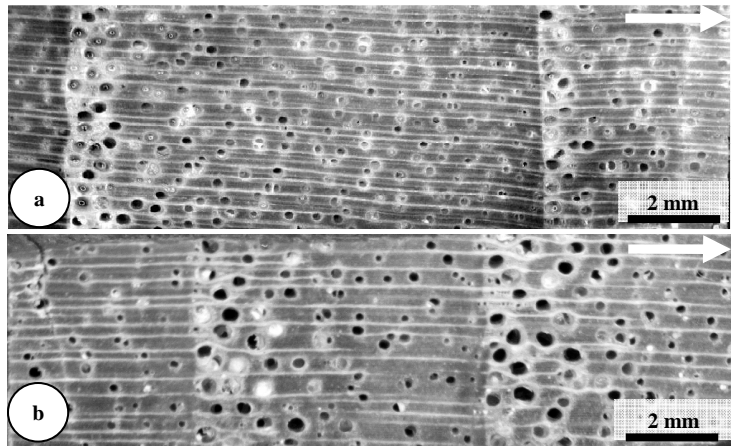


Plate 2.1 Transverse sections of ring-porous *Peronema canescens*. (a) Sample collected from Mata Ayer Forest Reserve, (b) Sample collected from Bukit Hari, Forest Research Institute Malaysia. Arrows indicate the direction of the bark side.

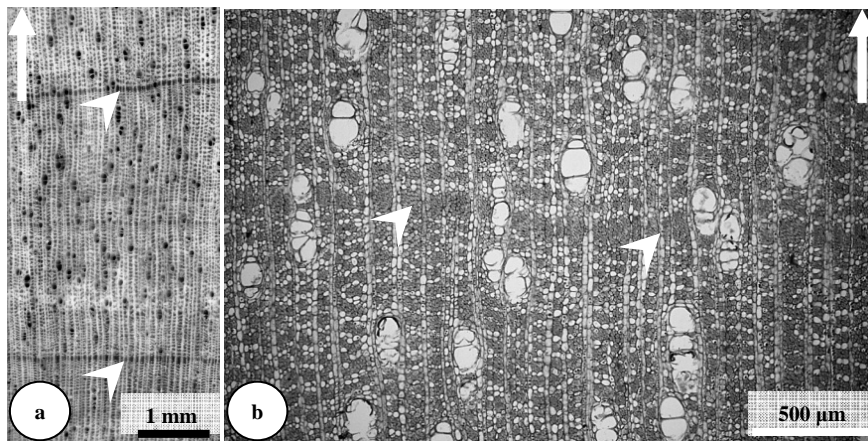


Plate 2.2 Transverse sections of *Paropsia vareciformis* showing growth ring boundary marked by fiber zone. (a) Arrowheads indicate darker and clear ground tissue of growth rings. (b) Arrowheads indicate fiber zones with few or no axial parenchyma and/or vessels. Arrows indicate the direction of the bark side.

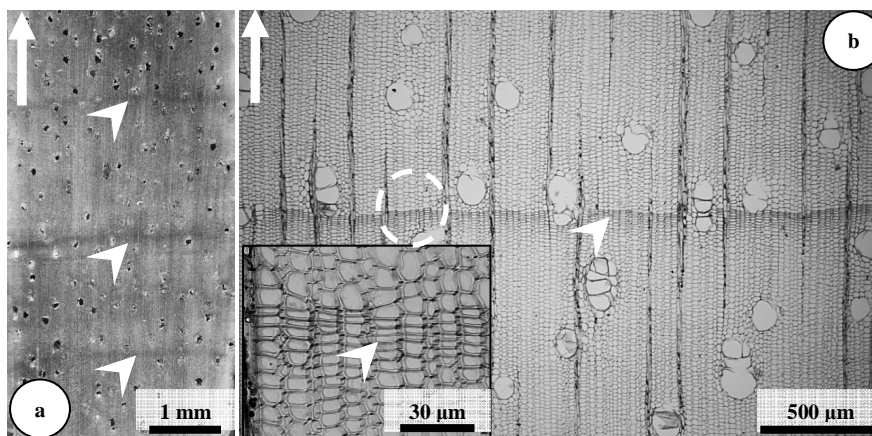


Plate 2.3 Transverse sections of *Litsea costalis* showing growth ring boundary marked by radially flattened fibers. (a) Arrowheads indicate darker ground tissue of growth rings. (b) Arrowheads indicate the changes in radial diameter of fibers from larger diameter in the beginning of growth to smaller diameter at the end of the growth ring. Inset image shows the enlargement (from dashed circle) of the radially flattened fibers (arrowhead). Arrows indicate the direction of the bark side.

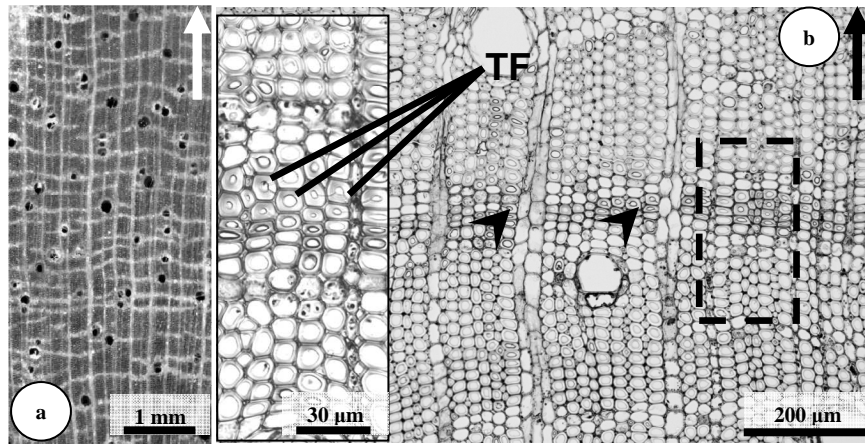


Plate 2.4 Transverse sections of *Garcinia urophylla* showing a growth ring boundary marked by thick-walled fibers. (a) No clear darker ground tissue was observed macroscopically. (b) Arrowheads indicate the differences in fiber wall thickness of the growth ring. Inset image shows the enlargement (from dashed box) of thick-walled fibers (TF). Arrows indicate the direction of the bark side.

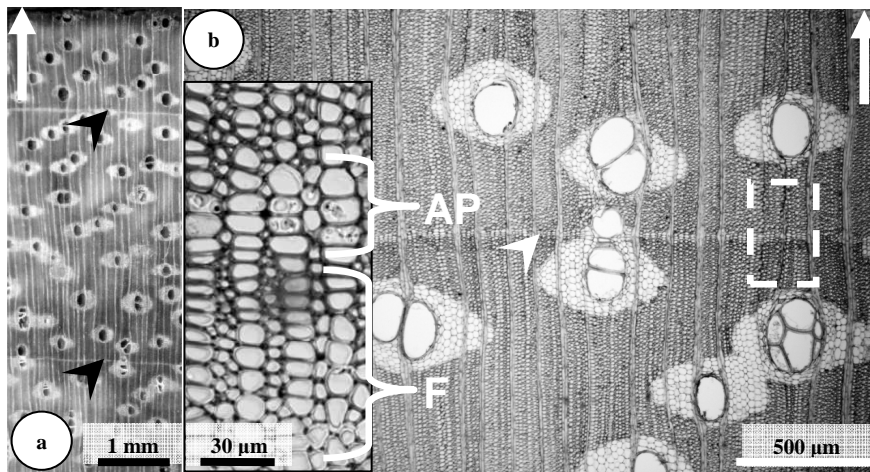


Plate 2.5 Transverse sections of *Intsia palembanica* showing a growth ring boundary marked by marginal parenchyma. (a) Arrowheads indicate the band of axial parenchyma. (b) Arrowhead indicates the marginal parenchyma band associated with the abrupt changes in fiber wall thickness and radial sizes. Inset image shows the enlargement (from dashed box) of axial parenchyma cells (AP) associated with abrupt changes in fiber wall thickness and radial sizes (F). Arrows indicate the direction of the bark side.

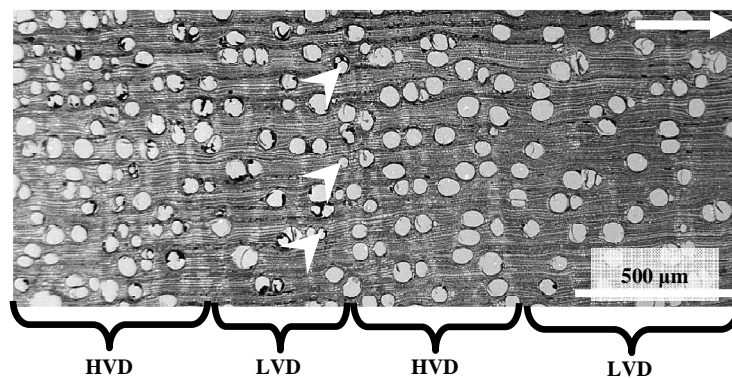


Plate 2.6 Transverse section of *Azadirachta excelsa* showing a growth ring boundary marked by weak changes in vessel diameter from large to small diameter (arrowheads) and vessel density from high (HVD) to low (LVD) density. Arrow indicates the direction of the bark side.

2.3.2 Irregularities in growth rings

Table 1 includes 17 species that showed poorly defined growth rings. IT was occasionally found in the xylem layer and was distinguished morphologically from normal growth boundaries by its sporadic character or circuit of non-uniform growth zones (e.g., Plate 2.7a, 2.7b & 2.7c). AR included growth rings that were indefinite, macroscopically as well as microscopically (e.g., *D. costulata*, Plate 2.8a & 2.8b). In the DC type, ring boundaries are discontinuous at one or more points along the circumference. This feature was well observed macroscopically (e.g., Plate 2.9). Another irregularity encountered was RTC in the parenchyma band. Minute observation revealed that there were differences between resin and traumatic canals with respect to the thickness of epithelial cell walls and the number of axial parenchyma cells surrounding these canals (e.g., Plate. 2.10b and 2.10d). Traumatic canals had thicker epithelial cell walls and were surrounded by more axial parenchyma cells than the resin canals.

A total of seven species showed IT irregularities: four species belonging to the MP group (*A. excelsa* in MAFR, *S. wallichii*, *T. wallichianus*, and *G. nervosa*), two belonging to the FZ group (*A. densiflora* and *D. malaccense*), and one belonging to the RF group (*L. costalis*). In this study, the AR irregularity type was the main problem in judging the growth ring boundary and was observed in 14 species. The majority of those species belonged to the FZ and RF groups; some also belonged to the VSD group (*A. excelsa* in MAFR and *G. arborea*). Most of the tree species with growth rings in the MP group showed DC irregularities (*A. excelsa* in AHFR, *T. wallichianus*, and *G. nervosa*). In this study, all the species belonging to Dipterocarpaceae formed concentric bands of resin canals (e.g., Plate. 2.10a and 2.10b), and *A. excelsa* and *S. wallichii* formed concentric bands with traumatic canals (e.g., Plate. 2.10c and 2.10d).

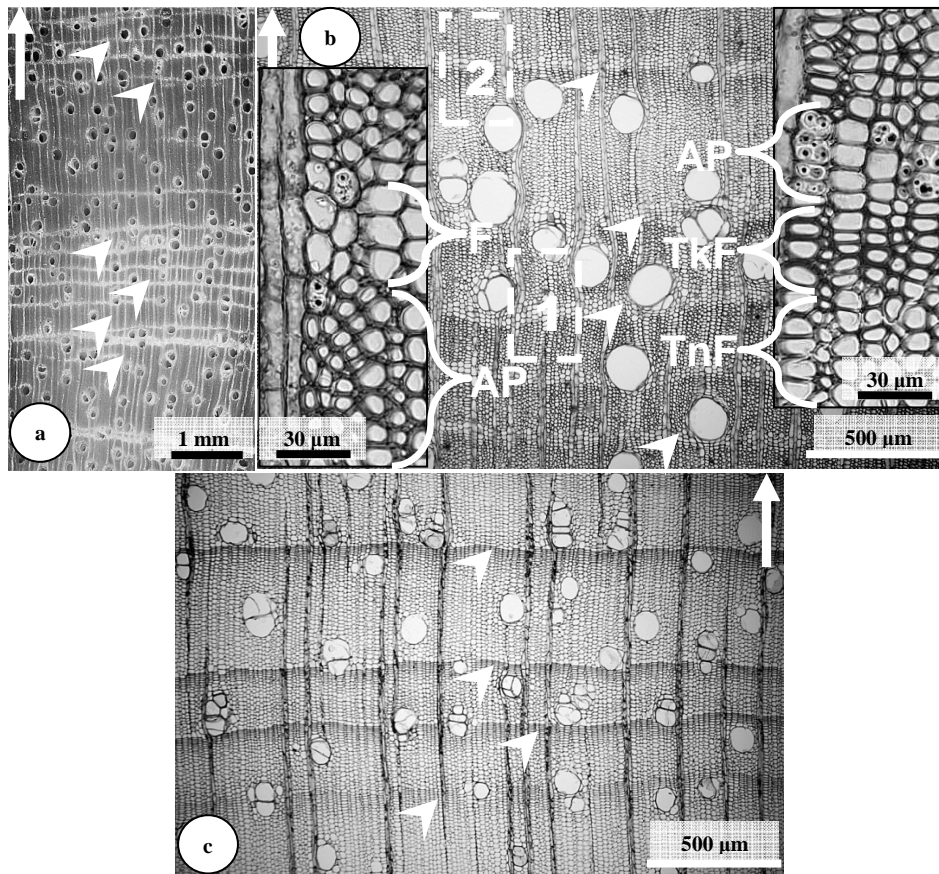


Plate 2.7 Transverse sections showing intermittent occurrence of growth ring boundaries in the xylem layer of *Azadirachta excelsa* (a and b) and *Litsea costalis* (c). (a) Arrowheads indicate the intermittent occurrence of axial parenchyma bands. (b) Arrowheads indicate the intermittent occurrence of marginal parenchyma in the xylem layer. Inset image 1 shows the enlargement (from dashed box 1) of axial parenchyma cells (AP) not associated with abrupt changes in fiber (F) wall thickness and radial diameter. Inset image 2 shows the enlargement (from dashed box 2) of axial parenchyma cells (AP) associated with abrupt changes in fibers from thin-walled (TnF) to thick-walled (TkF) fibers. Marginal parenchyma from dashed box 2 may be used as a growth ring boundary. (c) Arrowheads indicate the intermittent occurrence of radially flattened fibers in the xylem layer. The exact growth ring boundary is still unknown and the tree may have formed many growth rings in a single year. Arrows indicate the direction of the bark side.

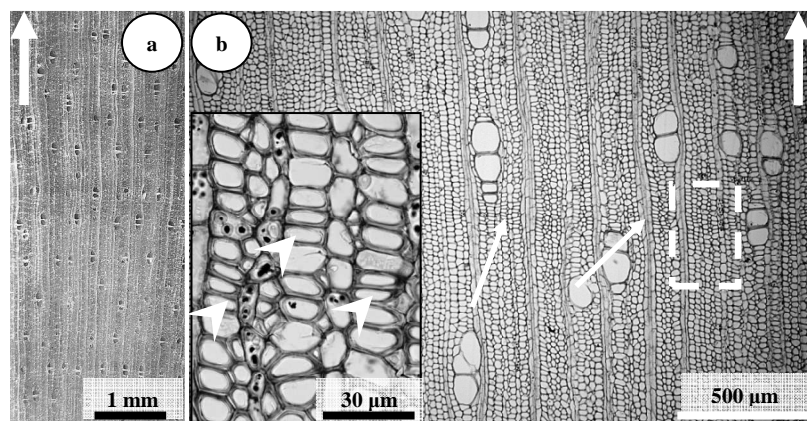


Plate 2.8 Transverse sections of *Dyera costulata* showing an ambiguous growth ring boundary. (a) No clear darker ground tissue can be observed macroscopically. (b) Thin arrows indicate radially flattened fibers. Inset image shows the enlargement (from dashed box) of radially flattened fibers (arrowheads) in the region of the ambiguous growth ring. Arrows indicate the direction of the bark side.

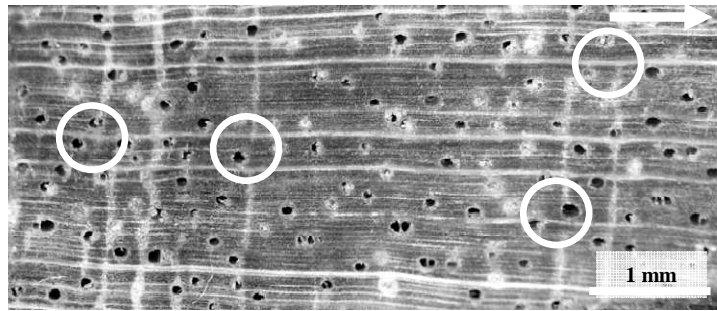


Plate 2.9 Transverse section of *Gironniera nervosa* showing discontinuous growth ring boundaries (circles). The parenchyma bands are either disconnected or disappear on the circuit. Arrow indicates the direction of the bark side.

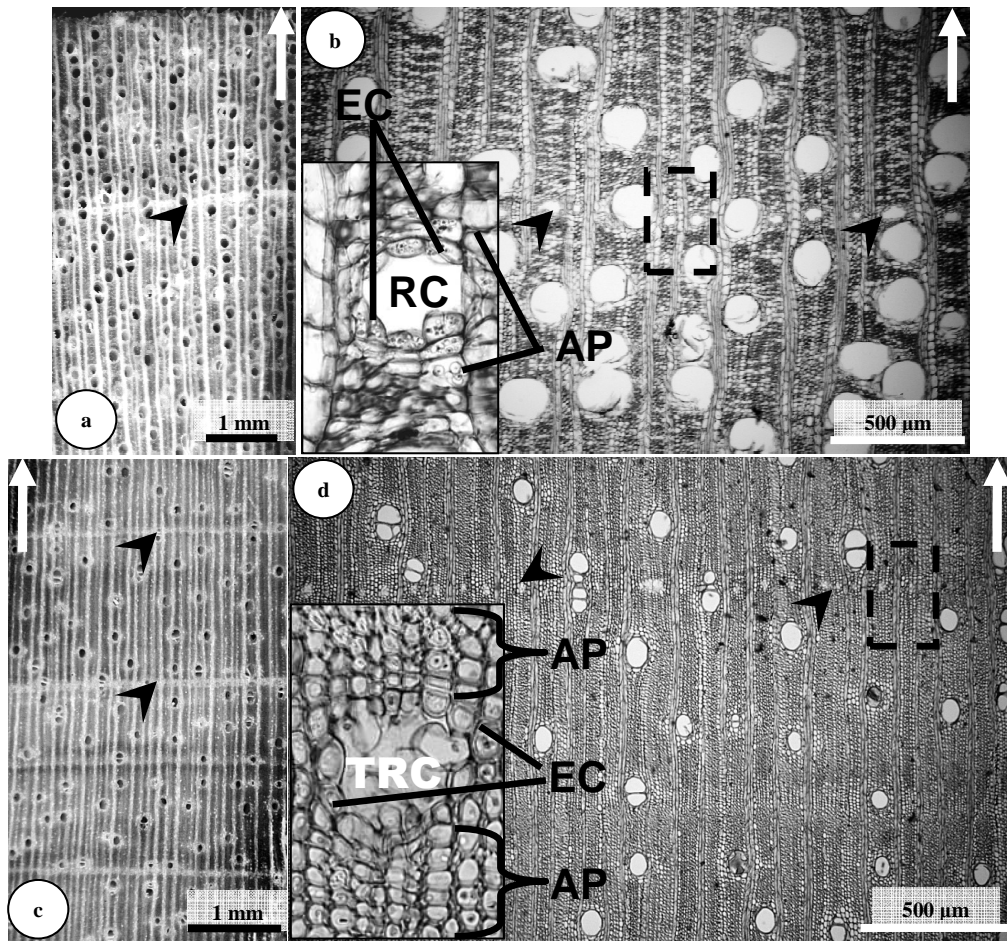


Plate 2.10 Transverse sections showing resin and traumatic canals in a parenchyma band. (a) Photomicrograph of *Hopea odorata*. Arrowhead indicates the parenchyma band. (b) Photomicrograph of *H. odorata*. Arrowheads indicate the concentric band of resin canal. Inset image shows an enlargement (from dashed box) of a resin canal (RC) surrounded by thin-walled epithelial cells (EC) and a few axial parenchyma cells (AP). (c) Photomicrograph of *Sindora wallichii*. Arrowheads indicate a series of parenchyma bands. (d) Photomicrograph of *S. wallichii*. Arrowheads indicate a concentric band of traumatic canals. Inset image shows an enlargement (from dashed box) of a traumatic resin canal (TRC) surrounded by thick-walled epithelial cells (EC) and massive axial parenchyma cells (AP). Arrows indicate the direction of the bark side.

2.4 Discussion

2.4.1 Occurrence of growth rings in tropical rainforest trees

The entire tree species studied had diffuse-porous porosity, except for ring-porous *P. canescens*. Most classifications of growth rings of temperate hardwood species in the literature are based on porosity, but it was not possible to judge the growth rings in most tropical rainforest trees. Even though most tropical rainforest trees do not form growth rings, mostly because there is less seasonality in tropical climates, this research proves that some of them do form growth rings, and that they are characterized by the anatomical features of FZ, RF, TF, MP, and variation in VSD.

Changes of fibers in the xylem were the most common feature to be characterized as a growth ring boundary in this study. In a zone in which the volumetric percentage of fibers is high, xylem is considered to function more in mechanical support than in water transportation and/or storage of carbohydrates. In species with the FZ type of growth ring boundary, the absence of vessels and/or axial parenchyma in the growth ring boundary was obvious. In the tropical rainforest climate, where precipitation and temperature are high throughout the year, we may assume that trees, in general, grow continuously and tree physiological activities continue to function. The author consider the appearance of fewer or no axial parenchyma cells and vessels in a xylem is attributable to the conditions under few carbohydrates to be stored and less soil water to be taken up during the time of their formation. Unexpected short drought may be one of such conditions.

Several species showed the growth ring boundary types of RF and TF. Faint dark tangential bands were observed macroscopically in some of the species. Using a microscope, the changes in the radial size of fibers and the thickness of the fiber wall close to the growth ring boundary was observed.

The formation of RF and TF may be explained through the lens of tree phenology. After observing darker rings on a disk of young para rubber (*Hevea brasiliensis*) grown in Malaysia, Ogata et al. (2001) found that the variation of fiber cell wall thickness and radial diameter of fibers in the darker rings were formed during the leafless period. Jalil et al. (1998) reported that such features are formed by the “wintering effect” during annual defoliation at the beginning of each year. Ogata et al. (2001) suggested that the RF and TF are formed at the time of defoliation rather than during the final phase of shoot elongation. It is important to investigate the disappearance of axial parenchyma and/or

vessels in the fiber zone and the changes of fiber radial size and cell wall thickness in order to better understand this group of growth ring boundaries.

There were five species with MP as the growth ring boundary. This feature was easy to observe macroscopically but still must be confirmed microscopically to avoid being confused with parenchyma bands, following the IAWA Committee (1989). The IAWA Committee (1989) recommended using the marginal parenchyma bands only when they were associated with abrupt changes in fiber wall thickness and/or radial sizes as growth rings. MP appears to function primarily to store starch. Dünisch and Puls (2003) observed in Meliaceae species that starch is predominantly stored in axial parenchyma and that soluble carbohydrates are mobilized at the beginning of the growing period, to support the formation of new leaves and secondary xylem before the new foliage produces carbohydrates through photosynthesis. Ogata et al. (2001) reported that banded parenchyma in mature *H. brasiliensis* grown in Malaysia are formed during the leafless period. In summary, the formation of MP could be best explained as starch storage for flushes of growth. In this study, therefore, the author considers MP to indicate the start of new growth, associated with an increase in fiber wall thickness and/or the decrease in fiber radial diameter. Fiber changes indicate the end of current growth in trees.

The author found clear VSD in *P. canescens* (MAFR and BHFRIM) and weak VSD in *A. excelsa* (BHFRIM and AHFR) and *G. arborea* (MAFR). The variation of vessel size and/or density is most likely caused by changes in water availability to trees (e.g., Baas 1976, Baas et al. 1983, Alves and Angyalossy-Alfonsi 2000, Ohashi et al. 2009). Ohashi et al. (2013) reported that vessel features varied independent of water availability in all species studied grown in rainforest climate and most species grown in savanna and monsoon climate. Ohashi et al. (2013) also stressed that even water availability influences variation in vessels for some trees in the tropics, it is also depend on the species characteristics such as vessel porosity, leaf phenology and drought adaptations, and internal factors such as phytohormones. However, the actual factor causing variation in vessel size and/or density in tropical trees are still in dispute.

Through microscopic observation of wood sections and information from the technical officers belonging to MAFR on general phenological observation of tree species and site conditions,

the author consider that VSD in *P. canescens* and *G. arborea* grown in MAFR were influenced by the genetic factor controlling vessel porosity, phenomena of leaf shedding and adaptation to 3 to 4 month's drought. VSD of *P. canescens* in BHFRIM was mostly considered to be influenced by the genetic factor since the site was along a river and always humid. *A. excelsa* grown in BHFRIM and AHFR were considered to be influenced by the genetic factor controlling semi-ring porous feature and short term leaf shedding. Future investigation on factors causing vessel changes in tropical rainforest trees should be conducted to deepen our understandings.

2.4.2 *Intra-specific variation in growth ring characteristics*

The growth ring characteristics of several species in present study differ from those reported in the literature (FRIM 1993, PROSEA 1994a, b, c). For example, FRIM (1993) described *D. costulata* as being marked by periodic layers without axial parenchyma, whereas PROSEA (1994b) described *D. costulata* as being marked by differences of fiber wall thickness. In this study, the author observed changes in fiber radial sizes as growth ring boundaries in *D. costulata* growing in BHFRIM and AHFR, and these samples were categorized as RF.

The author present the following reasons for the differences: (1) most of the findings in the literature were intended for timber use, and so accurate descriptions of each species were unnecessary, (2) the trees examined were grown under different climatic conditions, and (3) the small number of sample replicates failed to cover the anatomical variations. Information provided by FRIM (1993), for instance, was used mostly for wood identification. They grouped different genera from the same family together in a single commercial timber group. Therefore, the information about growth rings in a particular trade group might actually refer to any of several genera within the group. Information provided by PROSEA (1994a, b, c) mostly covered the tree species grown in southeast Asia, and thus the growth ring information from this source might come from trees grown in a tropical seasonal area.

Different climatic and site conditions also caused different growth boundaries to develop in a single species. *A. excelsa* growing in MAFR had MP as the growth ring boundary, *A. excelsa* growing in BHFRIM showed weak VSD, and *A. excelsa* growing in AHFR had a combination of MP and weak VSD. *A. excelsa* in MAFR was growing in a tropical monsoon climate and sheds leaves over a 2–3-

month dry period, resulting in the formation of MP as its growth ring boundary. *A. excelsa* in BHFRIM grows on a hill plot. Water availability during some low precipitation periods was distorted, perhaps triggering weak VSD in growth rings. In AHFR, the *A. excelsa* tree was growing in an open field. AHFR experienced at least 2 months of low precipitation, during which the tree continuously sheds leaves. Nobuchi et al. (1995) observed bands of parenchyma with small-diameter vessels as a growth ring in *H. odorata* in Thailand, and found that the growth rings were formed in the dry season. In present study, *H. odorata* grown in all study sites did not display distinct growth rings. Thus, the author suggests that the species reacts to environmental stressors, producing different growth ring boundaries.

2.4.3 Irregularities in poorly defined growth rings

In this study, irregularities were classified as IT, AR, DC, or RTC. The most common problems in dendrochronological studies in temperate and tropical seasonal areas are false rings and wedging rings (e.g., Worbes 1985, 2002, Bhattacharyya et al. 1992, Brienen and Zuidema 2006, Rozendaal and Zuidema 2011, Worbes and Fichtler 2011). These features, also known as discontinuous, partial, or incomplete rings, were classified as DC in this study.

The irregularities IT, AR, and DC may be attributed to unusual cambial activity at certain positions of the stem. The explanation for wedging rings given by Schweingruber (1988) and Worbes and Fichtler (2011) is that they develop when a tree changes its direction of growth relative to the light source due to competition with surrounding trees. This direction change probably leads to differences in the local supply of carbohydrates, water, mineral elements, and phytohormones (Dünisch et al. 1999). Other explanations for wedging rings are unusual climatic extremes or traumatic events. In the case of IT in species that have MP as their growth rings, the author differentiated the actual growth ring boundaries from the other parenchyma bands by strictly using the MP associated with abrupt changes in fiber wall thickness and/or radial size as the growth ring boundaries, following the IAWA Committee (1989).

The author identified RTC in parenchyma bands for some species. Macroscopic observation alone suggested that the growth ring would be featured by MP. Minute microscopic observation

revealed that in some cases, these tree species also produced parenchyma bands associated with resin canals and/or traumatic canals. Even though these canals became one of the main features that assisted in wood identification, their occurrence is considered to be contingent. The author recommends avoiding the use of concentric parenchyma bands with resin canals and/or traumatic canals as growth ring boundaries; as such canals are stimulated by traumatic events, such as physical or mechanical injuries.

2.5 Summary

This study confirmed that some tropical rainforest tree species do form growth rings. Anatomical features that were considered as growth ring boundaries were fiber zones, radially flattened fibers, thick-walled fibers, marginal parenchyma, and variation in vessel size and/or density. *Peronema canescens* showed well-defined growth rings, featuring ring-porous porosity characteristics. *Intsia palembanica* also showed well-defined growth rings, featuring marginal parenchyma. A total of 17 species showed indefinable growth rings when some irregularities were included, such as intermittent growth rings, ambiguous growth rings, discontinuous growth rings, and resin and/or traumatic canals in the parenchyma band. Growth rings were absent from nine of the studied species. These results confirmed the importance of selecting species that produce well-defined growth ring boundaries in tropical dendrochronology.

Chapter 3

Application of cambial marking with electrical stimulus and stable carbon isotope analysis to tropical rainforest trees

3.1 Introduction

Investigating the occurrence of growth rings by anatomical approach becomes a useful method when they are visible. There were studies detecting periodicity of wood formation in ringless species by isotopic analysis (e.g. Poussart et al. 2004, Verheyden et al. 2004, Ohashi et al. 2009). However, all those studies were conducted in tropical seasonal forest.

Anatomically, to ensure the study of wood formation in a known period by cambial marking, it is important to 1) minimize the wound response and 2) deduce the exact position of marked cambium. Based on past research, the author considered to apply cambial marking with electrical stimulus introduced by Imagawa and Ishida (1981) to present study. Succeeded marked cambia were then used as for isotopic analysis.

Imagawa and Ishida (1981) studied the influenced of electrical stimulation on xylem cells. In their first trial, they applied electric currents to *Abies sachalinensis*. The electric sources were direct current (DC) from dry batteries (1.5, 67.5, 135, and 337.5V) and direct current rectified from alternating current (AC–DC) using rectifiers (12V). As for electrodes, they used setting pins or sewing needles and spaced them vertically from 1 cm to 40 cm apart and immediately pulled out. They applied the electrical stimulation only once for each electrodes, from 5 seconds up to 2 hours. They concluded that the stimulation with DC 135V for 5 seconds did inscribe the xylem, featured by radially crushed tracheids. Imagawa and Ishida (1983) applied the electrical stimulation to broad-leaved trees, *Alnus japonica* and *Cinnamomum camphora* grown in subtropic region. They applied DC 154V for 5 seconds on 5 cm apart of dressmaker pins electrodes only once, and immediately removed the electrodes. They concluded that among the influenced cells, the tangential series of crushed cells seem to be most suitable mark represent as the position of enlarging cells at the time of marking. Note that, the crushed cells were not the cambial cells but enlarging cells which did not

complete the formation of secondary wall. Therefore, the author considers that similar feature can be observed in tropical rainforest trees.

The use of stable carbon isotopes in plant material in general, and tree rings in particular, has become wide spread when a good model became available predicting environmental effects on isotopic ratios ($\delta^{13}\text{C}$) (Farquhar et al. 1982, 1989). Discrimination against ^{13}C during photosynthetic carbon dioxide (CO_2) assimilation in C_3 plants decreases with decreasing ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a). This denotes increasing stomatal limitation of photosynthesis caused by either a decrease in stomatal conductance (g_s) and/or an increase in photosynthetic rate (A). The resulting increased intrinsic water use efficiency (A/g_s) is thus associated with isotopically enriched plant carbon (increased $\delta^{13}\text{C}$). Drought typically increases stomatal limitation of photosynthesis, whereas shading reduces it, thus showing corresponding excursions in $\delta^{13}\text{C}$ (Francey and Farquhar 1982).

Studies of stable carbon isotope composition in tree rings are growing in order to infer environmental conditions and tree physiological responses (McCarroll and Loader 2004). However, the absence of visible growth rings in most tropical trees so far excluded the application of this method in the tropics. Some reports show that growth boundaries can indeed be formed in the wood of tropical trees when a dormancy period is induced by a dry season (Worbes 2002). Deciduous species grown in seasonal climate are most likely to form distinct rings, but evergreens may also do so (Brienen and Zuidema 2005). Environmental stresses other than drought have also been shown to induce an annual pattern in wood structure, such as flooding and seasonally high salinity (Schöngart et al. 2002, Verheyden et al. 2004a). The identification of distinct rings in deciduous trees have furthermore opened the possibility to use stable isotopes for deriving past environmental responses (e.g. Poussart et al. 2004, Verheyden et al. 2004b, Hietz et al. 2005, Brienen et al. 2011). However, most of evergreen tropical trees that grown in the humid tropics do not form distinct growth rings (Jacoby 1989). This is a serious shortcoming since it precludes a dendrochronological approach and the use of stable isotopes for retrospectively analyzing the effect of environmental factors on tree growth.

In this chapter, the author discusses the effectiveness of cambial marking with electrical stimulation, combined with isotopic analyses in selected tropical rainforest trees grown under tropical monsoon and rainforest climates.

3.2 Materials and Methods

3.2.1 Research sites and samples

Research sites and sample species are the same as those in Chapter 2, five tree species, belonging to three families, planted in tropical monsoon Mata Ayer Forest Reserve (MAFR), five tree species, belonging to four families, planted in tropical rainforest Bukit Hari FRIM (BHFRIM), and 26 tree species, belonging to 18 families that grow naturally in tropical rainforest Ayer Hitam Forest Reserve (AHFR).

3.2.2 Cambial marking with electrical stimulation

Electrical stimulation was applied in selected tree stems using a portable device, namely Direct Current High Voltage Pulse Generator (DCHG). DCHG (Hokusetsu Systems, Sakai, Japan) used 4AA batteries as the power source. Source voltage can be boosted through a DC–DC circuit to higher voltages, namely 300, 400 and 500V, and shorter conducting time which is 0.5 second.

At breast height of the stems, two electrodes were inserted through the outer and inner bark into the mature xylem in a vertical orientation and fixed. Three set of electrodes inserted were spaced 5 cm longitudinally and 10 cm tangentially apart. As the electrodes, stainless-steel nails (0.12 mm in diameter, 1.51 cm in length) were used. Electrical stimulation consisting of 300, 400 and 500V were applied on three different set of fixed electrodes.

Marking of cambium with DCHG was applied two times at the same electrodes, in July 2011 and December 2011. In this chapter, December's marking were examined to discuss the trees' response to high DC pulse. The exact dates of marking were, December 12, 2011 in two individuals of all species in MAFR, December 27, 2011 in one individual of all species in AHFR, and December 28, 2011 in two individuals of all species in BHFRIM. Only one tree was sampled for each species for microscopy.

All wood samples contained December's marking were collected seven months later. Since mass-destructive sampling (i.e. felling of trees) was not allowed, wood blocks of 5 cm thick, 7 cm wide and 3 cm depth were collected using handsaw and chisel. The exact dates of sampling were on July 4–9, 2012 for those in AHFR (radial growth = 190–194 days), July 9, 2012 for trees in BHFRIM (radial growth = 193 days), and July 11, 2012 from trees in MAFR (radial growth = 199 days). Sampled wood blocks were directly fixed in 3% glutaraldehyde. As for light microscopy, transverse and radial sections of 20–30 µm in thickness were cut using a sliding microtome and double-stained with safranin and fast green. All sections were observed under an Olympus BX50 compound microscope.

In this chapter, the author discussed mainly on DC-influenced anatomical features and species-voltage suitability and evaluated the effectiveness of electrical stimulation as a tool for investigating wood formation in tropical rainforest trees.

3.2.3 Carbon isotope measurement

Species for preliminary carbon isotope measurement were selected based on the degree of growth ring distinctiveness, as mentioned in Chapter 2. In MAFR, *Peronema canescens* (well-defined growth ring, PCMA8), *Azadirachta excelsa* (poorly-defined growth ring, AEMA5) and *Hopea odorata* (growth ring absent, HOMA3) were selected. In BHFRIM, following the order of distinctiveness, same species were selected (PCBH9, AEBH8 and HOBH10). Meanwhile in AHFR, *Litsea costalis* (poorly-defined growth ring, LCAH1), *A. excelsa* (AEA2) and *H. odorata* (HOAH1) were selected.

A radial strip of 5 mm wide, 5 mm thick and 20 mm long were cut from December's marked blocks. Tangential sections of 0.1 mm in thickness were cut out from the strips with a sliding microtome. Depend on species, tangential sections were cut from cambium until marked position, at least 140 sections per species.

Holocellulose was prepared from wood sections by the Jayme-Wise method (Green 1963) with some modifications as reported by Leavitt and Danzer (1993). The extraction process removed the glutaraldehyde and made the sample homogenous. Holocellulose of 1–2 mg were obtained from

each sample sections, which was sufficient for carbon isotope measurement. In a series of sections prepared from each tree, only samples with odd numbers were measured to reduce time and effort.

Carbon isotope composition of holocellulose (ca. 1 mg) was measured with an isotope ratio mass spectrometer (Finnigan Delta S) connected to an elemental analyzer (NA2500) via an interface (Conflo II). As for reference materials, DL-Alanine ($\delta^{13}\text{C}_{\text{VPDB}} = -25.36\text{‰}$), L-Alanine ($\delta^{13}\text{C}_{\text{VPDB}} = -19.04\text{‰}$), Glycine ($\delta^{13}\text{C}_{\text{VPDB}} = -34.92\text{‰}$), and L-Leucine ($\delta^{13}\text{C}_{\text{VPDB}} = -29.06\text{‰}$) were used. Results were expressed using the conventional δ notation relative to the VPDB (Vienna Pee Dee belemnite), which is an internationally accepted reference material. The overall analytical uncertainty estimated by repeated analyses (10 replicates) of the DL-Alanine was within $\pm 0.1\text{‰}$ (SD).

3.3 Results

3.3.1 *Effect of cambial marking with electrical stimulation*

It was considered that cambium in all studied species were active at the time of marking. By the application of electrical stimulation, some of the cells were influenced depending on the degree of electrical stimulus. Table 3.1 summarizes the visibility of tangential series of influenced cells and type of influenced cells based on degree of electrical stimulus

Depending on degree of electrical stimulus, each species studied showed different response. With regards to marking or making inscription on xylem, the crushed cells (CC) are most noticeable among the influenced cells (e.g. Plate 3.1b–d). One or two cells at almost the same position in each radial file of cells were radially collapsed, so that the crushed cells were almost arranged in tangential series, thus represented a line macroscopically. Dependence of the degree of electrical stimulation, the visibility of tangential line was different among species studied (Table 3.1). Since they were nearly completely crushed, some of their lumina could not be found microscopically (e.g. Plate 3.1a & b). Tangential series of crushed cells were remarkable after inscribed by 500V (e.g. Plate 3.1d), almost in all studied species. The walls of crushed fibers and/or axial parenchyma cells were considerably thin and not fully lignified based on staining results with safranin and fast green.

Table 3.1 Visibility of tangential series and influenced cells inscribed by electrical stimulation.

Study Site / Family / Species	Degree of electrical stimulus						Suggested voltage for precise anatomical observation
	300V		400V		500V		
	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	
Mata Ayer Forest Reserve							
Dipterocarpaceae							
<i>Hopea odorata</i>	C	RTC	B	CC, ER, CV, RTC	A	CC, CD, ER, CV, RTC	400V
<i>Shorea roxburghii</i>	*Error	–	*Error	–	*Error	–	*Error
Meliaceae							
<i>Azadirachta excelsa</i>	C	CC, CD	B	CC, CD, ER, CV	A	CC, CD, ER, CV	500V
Verbenaceae							
<i>Gmelina arborea</i>	C	CC	B	CC, CD, ER, CV	A	CC, CD, ER, ER	400V
<i>Peronema canescens</i>	C	–	C	CC, CD, ER	B	CC, CD, ER, CV	500V
Bukit Hari FRIM							
Apocynaceae							
<i>Dyera costulata</i>	B	CC, ER	B	CC, ER, CV	A	CC, ER, CV	300V
Dipterocarpaceae							
<i>Hopea odorata</i>	B	CC, CD, RTC	A	CC, CD, ER, CV, RTC	A	CC, CD, ER, CV, RTC	400V
<i>Shorea roxburghii</i>	*Error	–	*Error	–	*Error	–	*Error
Meliaceae							
<i>Azadirachta excelsa</i>	C	CC, CD	B	CC, CD, CV, ER	A	CC, CD, CV, ER	500V
Verbenaceae							
<i>Peronema canescens</i>	C	–	C	CC, CD, ER	B	CC, CD, CV, ER	500V
Ayer Hitam Forest Reserve							
Apocynaceae							
<i>Dyera costulata</i>	B	CC, ER	B	CC, ER	A	CC, CD, ER, CV	400V
Clusiaceae							
<i>Garcinia urophylla</i>	C	CC	B	CC, ER	B	CC, CD, ER, CV	500V
Dipterocarpaceae							
<i>Hopea odorata</i>	B	CC, CD, ER, RTC	A	CC, CD, ER, RTC	A	CC, CD, ER, CV, RTC	400V
<i>Shorea acuminata</i>	*Error	–	*Error	–	*Error	–	*Error
<i>Shorea parvifolia</i>	B	CC, CD, ER, RTC	A	CC, CD, ER, CV, RTC	A	CC, CD, ER, CV, RTC	400V

Table 3.1 (continued)

Study Site / Family / Species	Degree of electrical stimulus						Suggested voltage for precise anatomical observation
	300V		400V		500V		
	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	
Ayer Hitam Forest Reserve							
Euphorbiaceae							
<i>Macaranga gigantea</i>	B	CC, ER	B	CC, ER, CV	A	CC, CD, ER, CV	400V
Lauraceae							
<i>Litsea costalis</i>	B	CC, ER	A	CC, CD, ER, CV	A	CC, CD, ER, CV	300V
Leguminosae							
<i>Intsia palembanica</i>	*Error	–	*Error	–	*Error	–	*Error
<i>Sindora wallichii</i>	B	CC, CD, ER	A	CC, CD, ER, TC	A	CC, CD, ER, CV, RTC	300V
Melastomataceae							
<i>Pternandra echinata</i>	C	–	B	CC, ER	B	CC, ER, CV	400V
Meliaceae							
<i>Aglaia oligophylla</i>	*Error	–	*Error	–	*Error	–	*Error
<i>Azadirachta excelsa</i>	C	CC, ER	B	CC, CD, ER	A	CC, CD, ER, CV	500V
Moraceae							
<i>Artocarpus scortechinii</i>	B	CC	B	CC, CD, ER	A	CC, CD, ER, CV	400V
<i>Streblus elongatus</i>	C	–	B	CC, CD, ER	A	CC, CD, ER, CV	400V
Myrtaceae							
<i>Syzygium polyanthum</i>	C	–	B	CC, ER	A	CC, ER, CV	500V
Olacaceae							
<i>Ochanostachys amentacea</i>	C	CC	B	CC, CD, ER	A	CC, CD, ER, CV	400V
Passifloraceae							
<i>Paropsia vareciformis</i>	C	–	C	CC, ER	B	CC, CD, ER	500V
Rhizophoraceae							
<i>Gynotroches axillaris</i>	C	CC, ER	C	CC, CD, ER	A	CC, CD, ER	400V
<i>Pellacalyx axillaris</i>	C	CC, ER	C	CC, CD, ER	B	CC, CD, ER	400V

Table 3.1 (continued)

Study Site / Family / Species	Degree of electrical stimulus						Suggested voltage for precise anatomical observation
	300V		400V		500V		
	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	Visibility of tangential series	Influenced cells	
Ayer Hitam Forest Reserve							
Rubiaceae							
<i>Aidia densiflora</i>	C	–	C	CC	B	CC, CD, ER	500V
<i>Diplospora malaccense</i>	C	–	B	CC, ER	B	CC, CD, ER	500V
<i>Timonius wallichianus</i>	B	CC, ER	B	CC, CD, ER	A	CC, CD, ER	400V
Sapindaceae							
<i>Pometia pinnata</i>	*Error	–	*Error	–	*Error	–	*Error
Sapotaceae							
<i>Palaquium maingayi</i>	C	–	B	CC, CD, ER	A	CC, CD, ER, CV	400V
Thymelaeaceae							
<i>Gonystlus confusus</i>	C	CC	C	CC, ER	A	CC, ER, CV	500V
Ulmaceae							
<i>Gironniera nervosa</i>	C	–	B	CC, ER	A	CC, CD, ER, CV	400V

*Error caused by the limitation of nail length and strength to penetrate through thick and hard bark. Electrical stimulus unable to inscribed the cambial and enlarging zone cells at the time of marking.

Visibility of tangential series: (A) Visible/strong, (B) Slightly visible/moderate, (C) Not visible/weak.

Influenced cells: (CC) Crushed cells, (CD) Cell deposits, (ER) Extended ray parenchyma, (CV) Crushed vessel element, (RTC) Occurrence of traumatic resin canal.

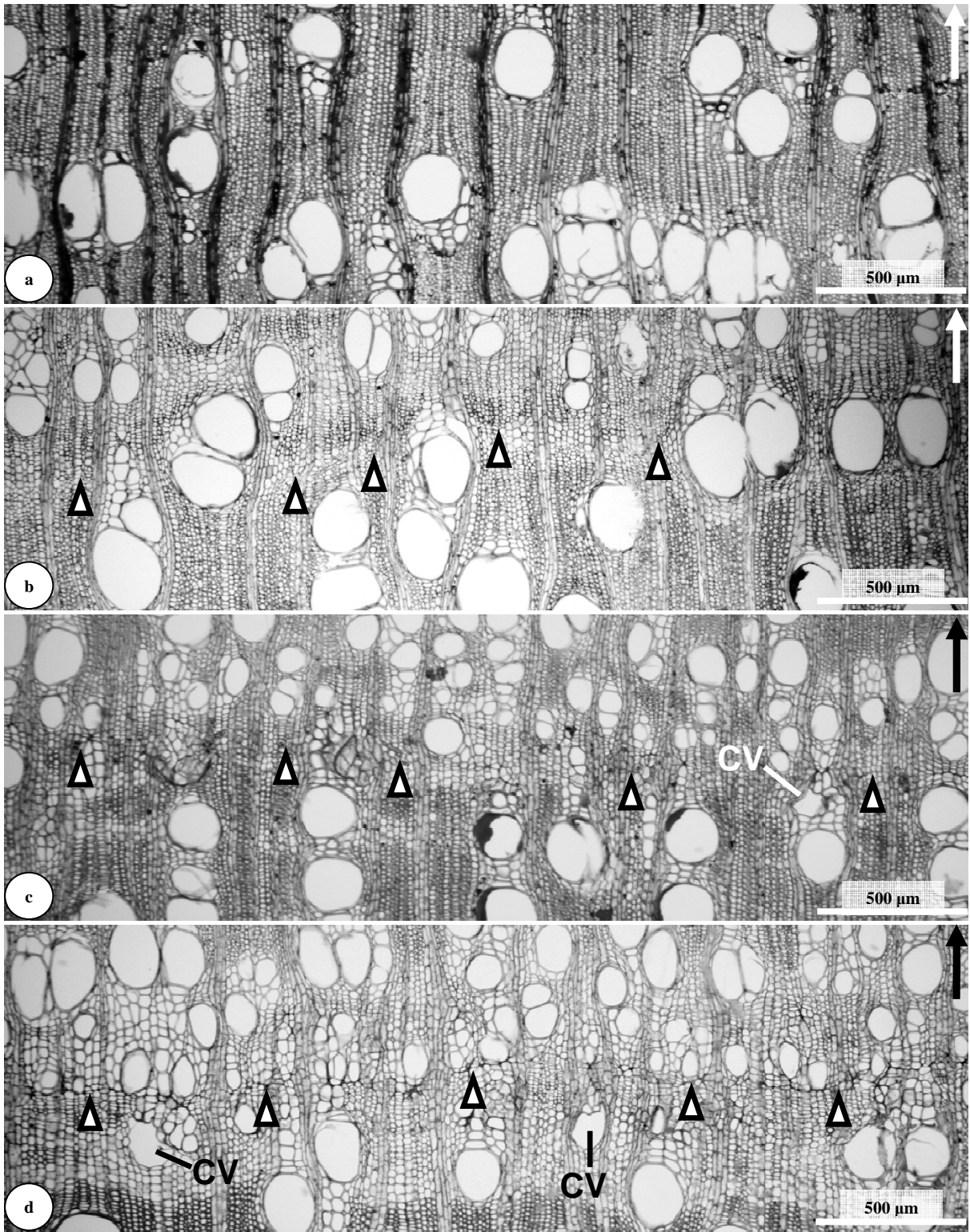


Plate 3.1 Transverse sections of *Azadirachta excelsa* show the different visibility of tangential line marked by electrical stimulus. **(a)** Normal xylem. **(b)** Xylem marked with 300V. **(c)** Xylem marked with 400V. **(d)** Xylem marked with 500V. Arrowheads indicate the tangential line made up by crushed cells. CV: crushed vessel element. Arrows indicate the direction of the bark side.

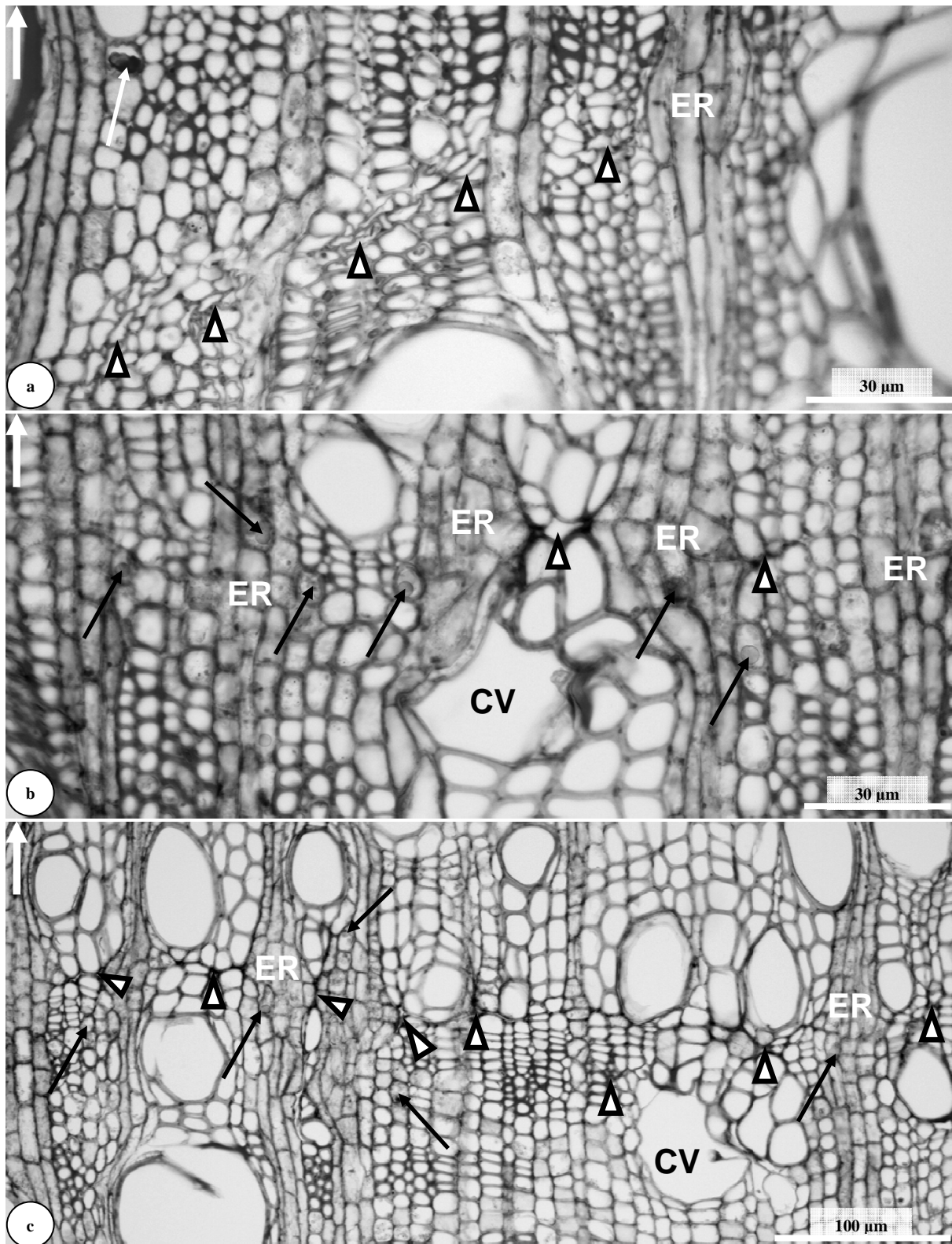


Plate 3.2 Transverse sections of *Azadirachta excelsa* show DC-influenced cells. (a) Enlargement of Plate 3.1b, showing radially crushed cells (arrowheads), extended ray parenchyma (ER) and deposit in cell (thin arrow). (b) Enlargement of Plate 3.1c, showing crushed cells (arrowheads), crushed vessel element (CV), extended ray parenchyma (ER), and deposit in cells (thin arrows). (c) Enlargement of Plate 3.1d, showing the radially crushed cells (arrowheads), crushed vessel element (CV), extended ray parenchyma (ER), and deposit in cells (thin arrows). White arrows indicate the direction of bark.

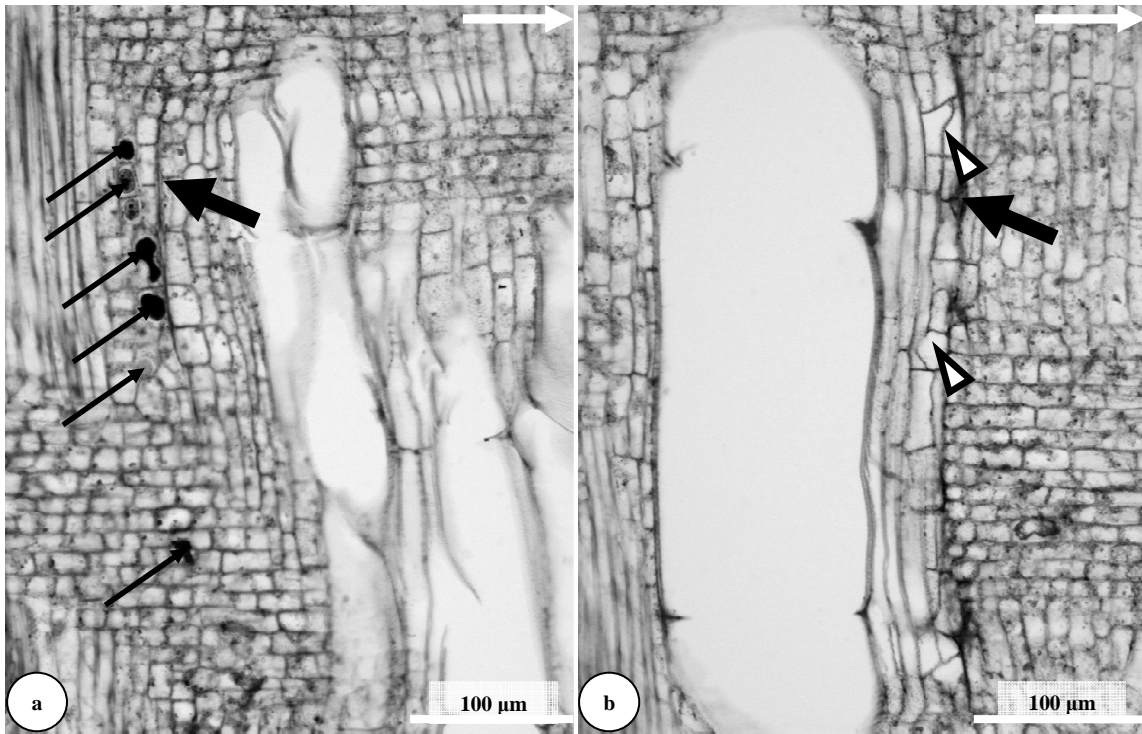


Plate 3.3 Radial sections showing influenced ray and axial parenchyma cells. (a) Deposits were included in axial and ray parenchyma cells (thin arrows) in *Azadirachta excelsa*. (b) Abnormal shape of axial parenchyma cells was observed (arrowheads) in *A. excelsa*. Large arrows indicate crushed cells. White arrows indicate the bark side.

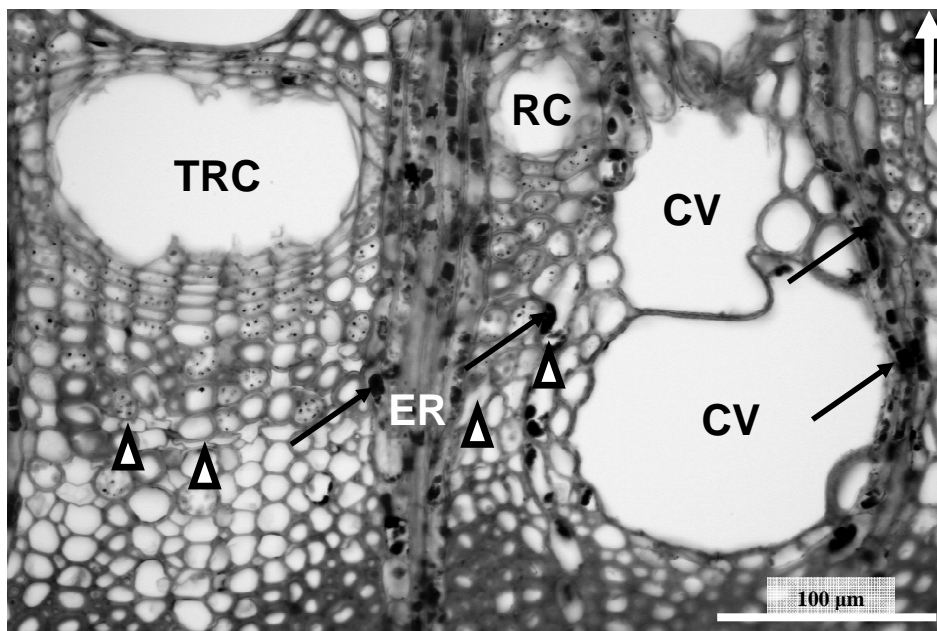


Plate 3.4 Transverse section of *Hopea odorata* shows the influenced cells. Arrowheads: crushed axial parenchyma cells, Thin arrows: Deposit in cells, ER: Extended ray parenchyma, CV: Crushed vessel element, RC: resin canal, TRC: traumatic resin canal. White arrow indicates direction of the bark side.

Although most of the CC seemed to be fibers and/or axial parenchyma cells judging from their features, a few of them were cells which were destined to become vessels (CV in Plate 3.1d and 3.2b). They were partially crushed radially. In most of studied species, most of CV can be found in 500V-marked and few of them in 400V-marked samples. The wall of CV was relatively thick and partially lignified based on staining results.

On the bark side of the crushed cells, two or three cells which were abnormal in shape and arrangement were found (e.g. Plate 3.1c & d). Based on radial section, most of them were strand-like (e.g. Plate 3.3a–b). Although it was not clear what they were differentiated from or to what they would eventually matured, they seem to be parenchyma-like cells. Almost normal shaped cells were relocated at the bark side of abnormal cells. In such regions, thin walled vessels were not found, but sizes changed from smaller to larger.

On the pith side of the crushed cells, thin walled cells were grouped in a tangential band. Most of these cells were considered to be fibers. Since their walls were relatively well stained with fast green, however, it was considered that they are not fully lignified. In species with thick walled fibers, such as *Hopea odorata*, *Shorea roxburghii* and *Garcinia urophylla*, the CC was considered to be the axial parenchyma cells because most of the thick fibers close to CC retained thickness. In some occasions, abnormal shape of thick fibers in those species can only be observed in 500V-marked samples. Some of the thin walled cells showed the cell deposits (CD) in their lumina. It was considered that such feature represent the residues of the protoplasm.

Rays were also profoundly affected by all degrees of electrical stimulation. Most of the ray parenchyma cells at the position adjacent to the CC were considerably expanded tangentially (e.g. ER in Plate 3.2a–c). It was considered that new ray parenchyma cells were added tangentially. CD was included in ray and axial parenchyma cells (e.g. Plate 3.2b &c, Plate 3.3a). It was considered that those CD were not always produced after the application of electrical stimulation, because it can also be observed in some of parenchyma cells at the pith side before marking (e.g. Plate 3.4).

Species belonging to Dipterocarpaceae and *Sindora wallichii* produced resin and/or traumatic canals (TRC) after the application of electrical stimulus (e.g. Plate 3.4). The cause of formation was unknown, either naturally formed or physiologically responded to electrode installation or physically

stimulated by DC pulse. The author considered that TRC were most probably formed later as a response to electrode installation because it was connected to the callus-like tissue formed by nail insertion. However, compared to marking results shown in Chapter 1, TRC in this study was not considered as serious problems since they were not overlapping with each other and did not disturb the anatomical investigation of wood formation.

3.3.2 Carbon isotope composition in selected tropical rainforest trees

Based on preliminary samples with 199-days of radial growth from marking position to the sampled cambium, *Peronema canescens* in MAFR (PCMA8) which represent the group of well-defined growth ring was not showed any distinct cyclicity of $\delta^{13}\text{C}$ values (Fig. 3.1a).

Growth ring boundary occurred at the distance of 3.6 mm from cambium. From this point, the values of $\delta^{13}\text{C}$ in PCMA8 gradually decreased to minimum and remained low until the end of periods, as a signal of active growing period. Poorly-defined growth ring of *Azadirachta excelsa* (AEMA5) was considered to show weak variations of $\delta^{13}\text{C}$ values (Fig. 3.1b). During 199-days growth, the $\delta^{13}\text{C}$ values decreased a little and increased towards the end of period. Compared to PCMA8, values of $\delta^{13}\text{C}$ in AEMA5 were slightly higher. Absent growth rings of *Hopea odorata* (HOMA3), however, showed variations of $\delta^{13}\text{C}$ values (Fig. 3.1c). Within 199-days radial growth, two small peaks appeared from DC-marked position to the end of the period. The minimum values of $\delta^{13}\text{C}$ in HOMA3 were similar as AEMA5. Based on the results, it can be considered that the studied species have variations of $\delta^{13}\text{C}$ values since those trees experienced 3–4 months dry period.

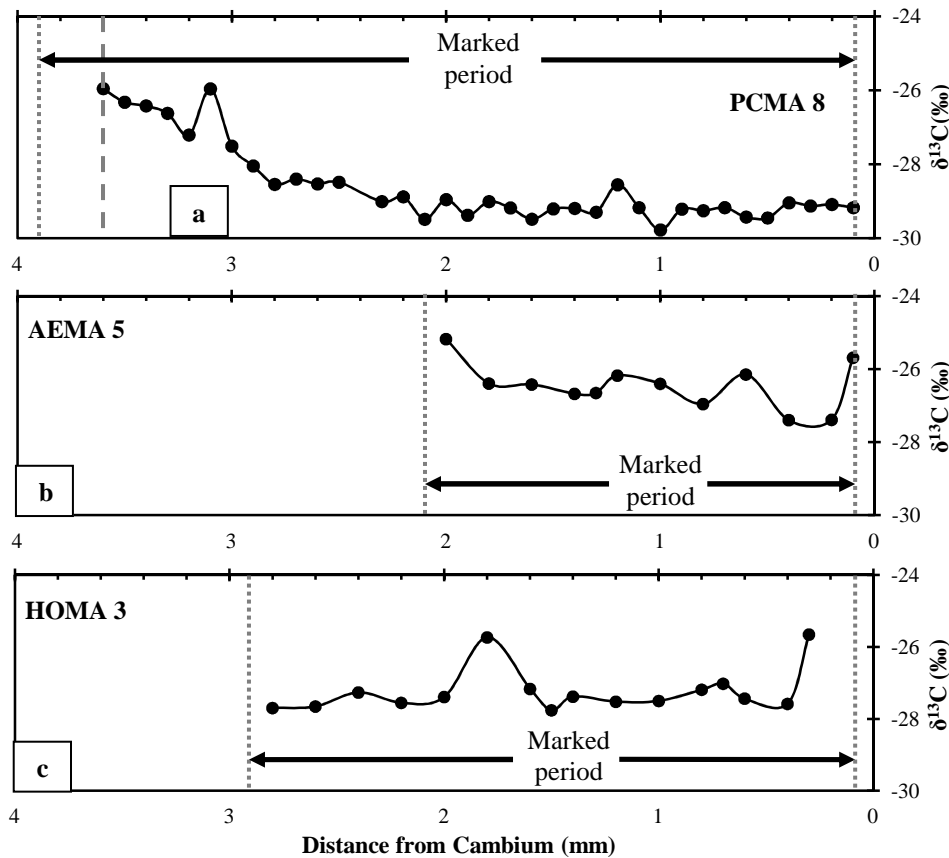


Fig. 3.1 Variations of $\delta^{13}\text{C}$ in (a) *Peronema canescens*, dashed line indicates growth ring boundary, (b) *Hopea odorata* and (c) *Azadirachta excelsa* grown in Mata Ayer Forest Reserve. The dotted lines indicate the position of cambial marking (Marked on Dec. 25, 2011). Samples were collected on July 11, 2012 (199-days radial growth).

Well-defined growth ring of *P. canescens* in BHFRIM (PCBH9) resulted similar variations of $\delta^{13}\text{C}$ values as in PCMA8 after 193-days radial growth (Fig. 3.2a). Unfortunately, the samples were measured until 2.6 mm from cambium because some of the samples were missing during the measurement. However, the point of 2.6 mm was located at the region of the end of pore zones. The values of $\delta^{13}\text{C}$ gradually decreased until minimum and again showed a small peak in between of small vessels region. After decreased, the $\delta^{13}\text{C}$ values remained low until the end of period. Compared to the values of $\delta^{13}\text{C}$ in PCMA8, the values were slightly higher, indicating a signal of weak drought in BHFRIM. *A. excelsa* (AEBH8) showed extraordinarily high values of $\delta^{13}\text{C}$ within 193-days growth (Fig. 3.2b). Although there were a decreased of $\delta^{13}\text{C}$ values, it was still high especially for trees grown in tropical rainforest climate. The possible reason is that the selected AEBH8 was growing on a hill slope. It was unknown whether the slope condition can be considered as drought condition. *H. odorata* (HOBH10) showed similar pattern of $\delta^{13}\text{C}$ variations as HOMA3, when there were two peaks were obtained within 193-days radial growth (Fig. 3.2c). The appearances of these peaks indicate that

HOBH10 might experience weak drought at some time. Based on the results obtained, it was considered that the studied species might or can experienced drought even they were grown under tropical rainforest climate.

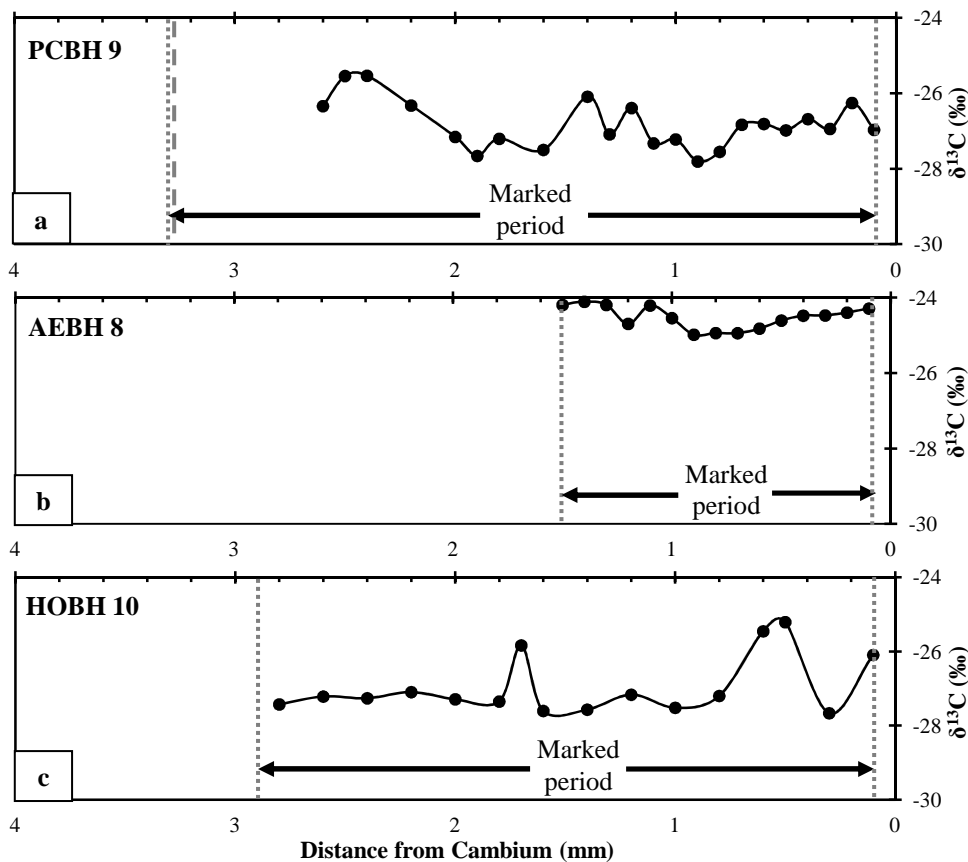


Fig. 3.2 Variations of $\delta^{13}\text{C}$ in (a) *Peronema canescens*, dashed line indicates growth ring boundary, (b) *Hopea odorata* and (c) *Azadirachta excelsa* grown in Bukit Hari FRIM. The dotted lines indicate the position of cambial marking (Marked on Dec. 28, 2011). Samples were collected on July 9, 2012 (193-days radial growth).

Poorly-defined growth ring species of *Litsea costalis* grown in Ayer AHFR (LCAH1) did not show any variations of $\delta^{13}\text{C}$ values during 190-days radial growth, but small increased in $\delta^{13}\text{C}$ values were detected. LCAH1 was grown close to the river and was considered to receive much amount of water. However, it was not presume that LCAH1 did not experience any drought such as increased in salinity. *A. excelsa* (AEA2) showed a variation of $\delta^{13}\text{C}$ values. During 194-days growth, values of $\delta^{13}\text{C}$ rapidly decreased and slightly increased to the end of period. AEA2 was grown on an open field and sometimes experienced flooding during the period of heavy rain. The evergreen *H. odorata* in AHFR (HOAH1) did not show any variations of $\delta^{13}\text{C}$ values when most of the values were constantly low within 194-days radial growth.

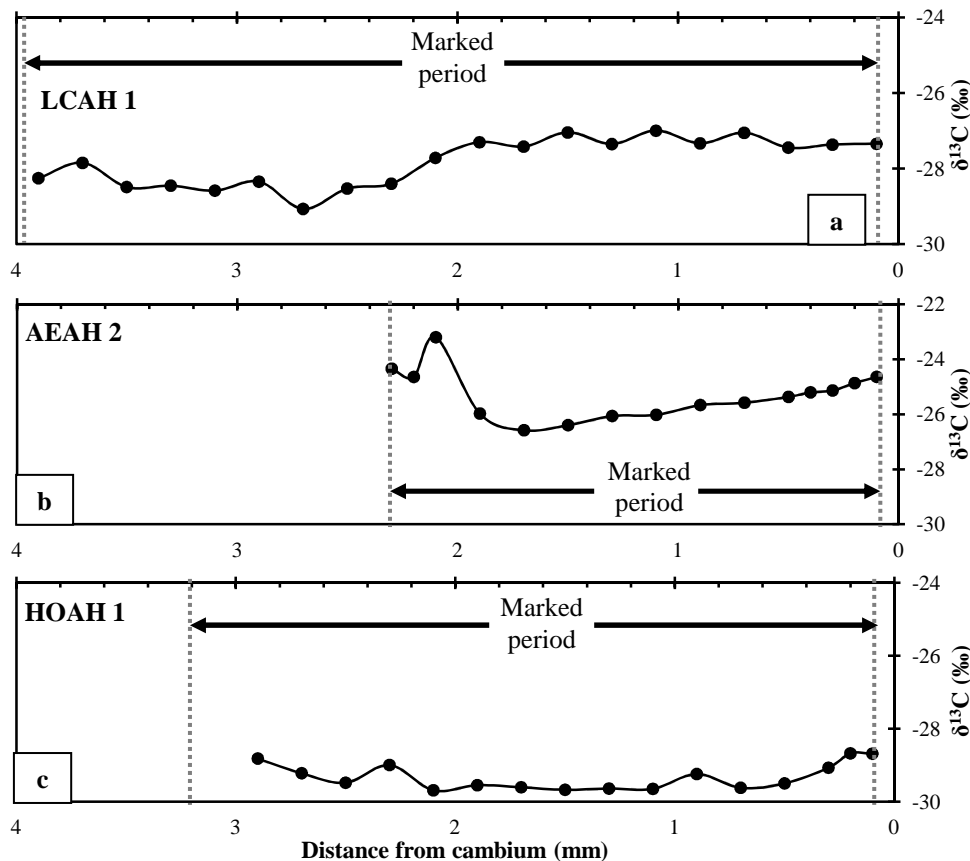


Fig. 3.2 Variations of $\delta^{13}\text{C}$ in (a) *Litsea costalis*, (b) *Hopea odorata* and (c) *Azadirachta excelsa* grown in Ayer Hitam Forest Reserve. The dotted lines indicate the position of cambial marking (Marked on Dec. 27, 2011). Samples were collected on July 5, 2012 for *L. costalis* (190-days radial growth) and July 9, 2012 for both *A. excelsa* and *H. odorata* (194-days radial growth).

3.4 Discussion

3.4.1 DC-influenced cells

In all species examined influenced cells such as the CC, CV, ER, and some TRC occurred during the 190-199 days radial growth. Judging from the state of their appearance, it was presumed that the differentiating cells were strongly influenced by the application of the electrical stimulation and consequently resulted in necroses or abnormal differentiations. The exact causes from which the necroses or abnormal differentiation resulted were not clarified in this study. Imagawa and Ishida (1982) reported that the electrical stimulation or current flows mainly through the cells near or in the cambium, in which protoplasm were abundantly contained. Based on the state of their appearances in the studied samples, therefore, it may be possible to assume that the enlarging cells were responded to the electrical stimulation and produced the influenced cells. It was also considered that both crushed cells and thin walled and unligified cells died during the course of their differentiation.

Among influenced cells, CC was located at the position nearest the marked cambium and had the thinnest walls. Therefore, they were assumed to be the cells immediately before the initiation of the secondary wall formation. They appeared to be radially collapsed by pressure which occurred after necroses because of their thinnest and weakest walls. As a result, since they were located at almost the same position in each radial file of cells, they were aligned in tangential series. Clearly, CC were most suitable feature to deduce the position of enlarging cells at the time of marking, because such tangential series or line of CC can be easily identified microscopically or macroscopically. Based on the degree of electric stimulus, every species showed different response when they show different visibility degree of tangential series. The differences were considered to arise from the differences of xylem cell organization. Imagawa and Ishida (1983) reported that the appearance of paratracheal parenchyma cells remarkably contributed to the tangential series of crushed cells.

Generally, the differentiation of a vessel element inclines to advance faster than the surrounding cells (e.g. Imagawa and Ishida 1972, Wakuta et al. 1973). In fact, the walls of the CV were relatively thick and more lignified compared to those of the surrounding cells. Imagawa and Ishida (1983) reported that vessels collapsed because of their large lumina. Thick and lignified walls may be not sufficient to maintain large volumes that would resist any pressure occurred after the necroses (Imagawa and Ishida 1983). Thin walled and unligified vessels surrounded by normal cells were observed on the bark side of CC in some species. Since such vessel initiated the differentiation to some extent and the surrounding cells were considered to have not begun, the vessel was left in an immature state and the surrounding cells were usually differentiated after the electrical stimulation. In a viewpoint of elucidation of vessel differentiation, the occurrence of CV and thin walled and unligified vessels were interesting to be investigated.

Occurrence of TRC in selected Dipterocarpaceae and some other species were expected because of their nature. However, the occurrence of TRC seems to be less and not overlapped when compared to mechanical cambial marking (i.e. knife marking). In the region with presumed DC-influenced cells were located (i.e. in between of two electrodes in longitudinal direction), TRC features were not as obvious as those in region close to electrodes insertion. Therefore, TRC in this

study was considered to be formed later after DC-marking because most of TRC observed were connected to callus tissue formed at the region of electrodes insertion.

The appearances of ER and abnormal shaped cells in some studied species did not resulted from the death of cells. It was considered that those feature derived from the unusual differentiation or unusual cell divisions may occurred. However, it was presumed that the cambial cells were sometimes not directly influenced by the electrical stimulation because the ER was directly recovered to normal ray parenchyma afterwards and the abnormal shaped cells were scarce.

It was showed that the marking of cambium with electrical stimulation in studied species was beneficial to study wood formation in tropical rainforest trees since the normal production of cells was expected after DC-stimulation.

3.4.2 *Application of carbon isotope measurement in tropical rainforest trees*

Based on the distinctiveness classification of growth rings discussed in Chapter 2, the author selected tree species belongs to well-defined, poorly-defined and absent growth ring groups in each research sites for testing the effectiveness of isotopic approach. Anatomically, growth ring features of those belong to poorly-defined and absent were not easy and impossible to be observed. Therefore, the author applied isotopic analysis to detect the periodicity of wood formation for each class of growth ring distinctiveness.

The preliminary studied samples collected in MAFR, BHFRIM and AHFR consisted of 190- to 199-days radial growth and not represent annual cyclic of $\delta^{13}\text{C}$ values. The values of $\delta^{13}\text{C}$ in all studied species showed a part of an annual cycle or some variations when the values of $\delta^{13}\text{C}$ in some species decreased to minimum and increased to maximum. Depend on research sites, the variation of $\delta^{13}\text{C}$ values were different. The variation of $\delta^{13}\text{C}$ values was considered as reflection to the seasonal changes of the physiological and environmental factors which affected carbon isotope fractionation. Poussart et al. (2004) reported that moisture availability is inversely related to the $\delta^{13}\text{C}$ value of tropical trees. Based on the general pattern of $\delta^{13}\text{C}$ values for each research sites, it was considered that MAFR had seasonality in moisture availability, followed by BHFRIM and later by AHFR.

However, the drought experience might be subjective for each site, depend on climate and site condition.

The values of $\delta^{13}\text{C}$ in well-defined growth rings species, *Peronema canescens*, were different than those belonged to poorly-defined (*Azadirachta excelsa* and *Litsea costalis*) and absent growth rings (*Hopea odorata*). PCMA8 showed gradual decreased to minimum and remain low until the end of the period. The pattern of rapid rise from just before the ring boundary followed by gradual or rapid decrease to the minimum were observed in temperate trees and can be attributed to the use of ^{13}C -enriched carbon reserve for xylem formation (Helle and Schlser 2004). The annual formation of growth rings in *Peronema canescens* grown in MAFR is unknown, but based on the information by technical staffs; they shed all leaves in the dry period. Other species did not shed until leafless in, but they start to flush new leaves while retaining some old leaves. When electrical stimulation was applied, the trees started to shed leaves. Based on anatomical observation of PCMA8, the DC-marked cells position was in the region of small vessels (i.e. latewood), approximately 0.3 mm from the position of current growth rings in the bark side. The gradual decreased values of $\delta^{13}\text{C}$ in PCMA8 were considered as a result of the use of some carbon reserves when entering the initial stage of xylem formation (i.e. large pore zones). The annual cyclicity of $\delta^{13}\text{C}$ values in the studied species were unpredictable yet. Therefore, it is important to analyze those cycle in future.

3.5 Summary

In this experiment, carbon isotope did not provide so much useful information on tree growth, but the DC-pulse marking was proved to be useful to create artificial indication in determining the time of growth ring formation. The DC-influenced cells such as crushed cells, crushed vessels, extended ray parenchyma, and in some occasion occurrence of abnormal-shaped cells are considered to be the most suitable features to deduce the position of enlarging cells at the time of marking. The variation of $\delta^{13}\text{C}$ values in preliminary studied species showed that isotopic approach can be applied and effective to deepen the understanding of wood formation.

Chapter 4

Radial growth periodicity in tropical rainforest trees

4.1 Introduction

Tree rings in woody plants are generally induced by seasonally alternating favorable and unfavorable growth conditions. Stress factors which occur seasonally in the tropics are dry seasons and flooding (Worbes 1995). Worbes (1992) indicated that an annual dry season with a length of 2 to 3 months and less than 60 mm monthly precipitation induce annual rings in tropical trees. The existence of two rings per year has been documented by Jacoby (1989) and Gourlay (1995) for trees grown in regions with dry seasons. These climatic stress factors induce an inactive cambium in trees and, in consequence, growth zones in the wood. As mentioned in Chapter 1, some tropical rainforest trees show seasonal rhythm of radial growth, phenological events and cambial activity revealed by dendrometer measurement, phenological observation and anatomical observation of cambium.

One of the ways to monitor cambial activity is through measurements of the electrical resistance of the cambial zone with a Shigometer (Shigo and Shortle 1985). Shigometer have occasionally been used to describe cambial activity (e.g. Torelli et al. 1990, Worbes 1995). Worbes (1995) reported that higher electrical resistance showed less cambial activity. In cellular level, Imagawa and Ishida (1982) reported that total number of cells in cambial and enlarging zones increased with the decreasing electrical resistances. However, Shigometer uses direct current (DC) voltage and measures the electric resistance with changing the position of trunk, and accordingly uncertainties were included in estimating the variation of cambial activity.

Unlike electrical resistance, electrical impedance comprises two components: a resistive (real) part and a reactive (imaginary) part. Practically, impedance is considered as the total resistance of an AC circuit. The conductive characteristics of tissue fluids provide the resistive component, whereas the cell membranes, acting as imperfect capacitors, serve as the frequency-dependent reactive component. When a low-frequency alternating current (AC) is applied to plant tissues, it flows through the apoplast, whereas its passage through the symplast is limited by the high impedance of the membrane. With increased AC frequency, membrane impedance decreases and the amount of current

that passes through the symplast increases (Cole 1968). Impedance measurements made over a range of frequencies therefore reveal information about the extra- and intracellular fluids.

Electrical impedance measurements have been used to estimate frost hardiness (e.g. Stout 1988a, 1988b, Repo 1992). In those studies, electrical impedance measurements provided a means of non-destructively analyzing variations in intra- and extracellular resistances and the condition of the membranes. Based on the concept of electrical resistance in cambium (e.g. Imagawa and Ishida 1982, Worbes 1995), it was assumed that impedance value of cambium will be smaller during growing season when external and internal resistance of tissues become smaller with increasing moisture and solutes in tissues.

In Chapter 2, the studied tree species have growth rings showing anatomical variation. Therefore, it is necessary to determine the seasonal rhythm of radial growth, seasonal phenological events and seasonal cambial activity in the current study.

4.2 Materials and Methods

4.2.1 Study sites and samples species

Radial growth and changes in phenological events were investigated in five tree species planted in Mata Ayer Forest Reserve (MAFR), five tree species planted in Bukit Hari FRIM (BHFRIM), and 26 tree species that grow naturally in Ayer Hitam Forest Reserve (AHFR). Ten individuals were selected for each species in MAFR and BHFRIM. Only two to three individuals were selected in AHFR, because they were sparsely distributed within one forest compartment, and hence were difficult to observe many trees. Results presented in this chapter were mostly results of species which represent one of the growth ring distinctiveness groups such as well defined, poorly-defined and absent growth rings in each study sites.

Climatic conditions during field experimental period in MAFR, BHFRIM and AHFR were shown in Fig. 4.1a, b and c. In MAFR, data collections were conducted from July 2011 to December 2012. There was no distinct dry period during the experimental period based on the pattern of precipitation. Highest precipitation was observed in August and September 2011 and the lowest was in July 2012. The total amount of precipitation was still low compared to BHFRIM and AHFR. Mean

temperature increased from January to February 2012 and continued until July 2012. In BHFRIM, field data collections were conducted from July 2011 to July 2012. High precipitation were recorded in BHFRIM during the experimental period, always exceeding 100 mm per month. Data collections in AHFR were conducted from December 2011 to December 2012. Compared to BHFRIM, AHFR showed some variation in precipitations when low precipitation was recorded from January to February 2012 and May to August 2012. Highest precipitation was recorded in November 2012.

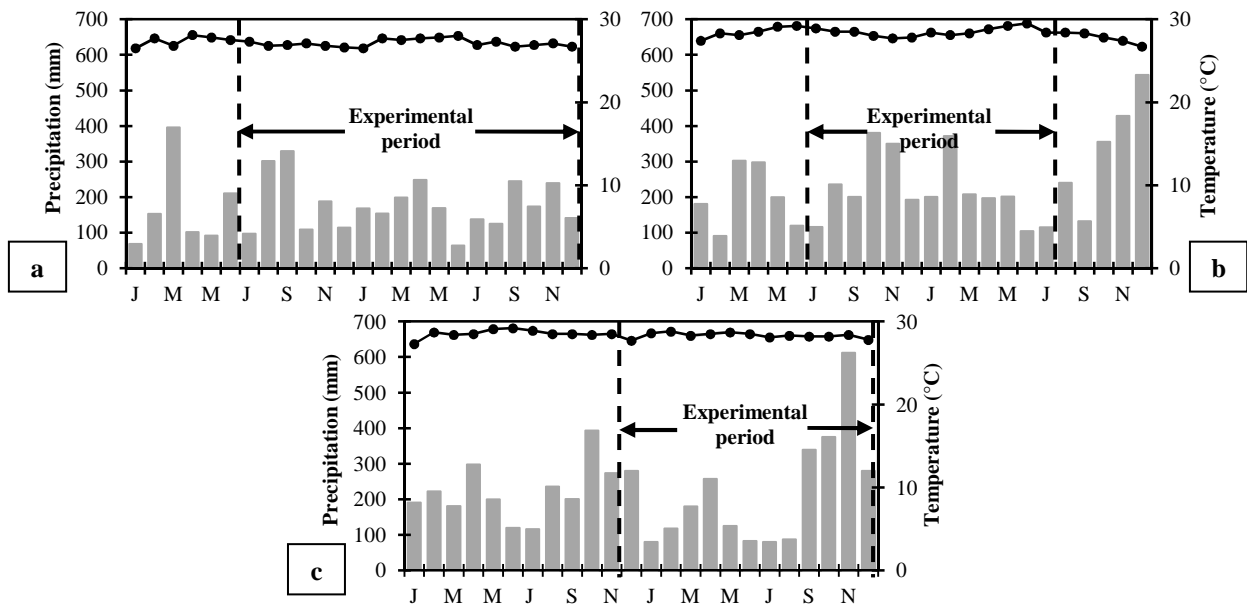


Fig. 4.1 Diagram of monthly total precipitation (bar) and mean temperature (line) of study sites from January 2011 to December 2012. (a) Mata Ayer Forest Reserve (MAFR). (b) Bukit Hari FRIM (BHFRIM). (c) Ayer Hitam Forest Reserve (AHFR). Dashed lines indicate experimental period. Climate data for AHFR was provided by Universiti Putra Malaysia, and data for MAFR and BHFRIM were obtained from the Malaysian Meteorological Department.

4.2.2 Dendrometer and impedance measurement, and phenological observation

Radial growth of each studied trees was measured with band-type dendrometer. Data collection was performed every two weeks from July 12, 2011 to December 18, 2012 in MAFR, July 14, 2011 to July 31, 2012 in BHFRIM, and December 14, 2011 to December 18, 2012 in AHFR.

Leaf phenology was observed with a binocular. Score of 0 (0%), 1 (less than 20%), 2 (20–80%), or 3 (more than 80%) was used in recording the cover percentage of mature leaves (green or dark green), old leaves (yellowish or brownish), leaf emergence (new leaf buds), and expanded leaves (new leaves expended from buds) on the crown for each individual tree.

Cambial activity of studied trees was monitored by measuring electrical impedance with a portable impedance measuring instrument (IMI, Hokusetsu Systems, Sakai, Japan). IMI has a rechargeable DC power source (rechargeable 4AA batteries). DC is converted to AC through a DC-AC inverter, and frequency of 1000Hz is applied to the sample with two fixed electrodes. Based on the value of applied frequency, relative changes of impedance values are detectable.

As for the electrodes, stainless-steel nails (0.12 mm in diameter and 1.5 cm in length) were used as the fixed electrodes and placed longitudinally 5 cm apart at breast height of a stem. The electrodes were inserted until it reached the xylem. Stainless wires were also fixed to the electrodes in case the nails were underneath tree bark after a certain period. Impedance values were measured repeatedly at the same position of trunk, on the same day as dendrometer measurement and phenological observation. Impedance measurement was conducted on the trees in BHRIM and AHFR only because the number of device was limited. Through impedance measurement, the absolute value of impedance is variable according to the installation of permanent electrodes as well as species characteristics. Low impedance values indicate the high cambial activity and high values indicate low cambial activity.

4.3 Results

4.3.1 Radial growth and leaf phenology of trees grown in MAFR

Peronema canescens in MAFR (PCMA) showed seasonal rhythm of radial growth (Fig. 4.2a). Based on phenological observation, PCMA showed seasonal leaf changes when leaf shedding occurred (Fig. 4.2b). PCMA showed active growth in the early stage of measurement until November 8, 2011. During this period, trees were covered with leaves and received much amount of precipitation. Radial growth slows down from November 22, 2011 until April 24, 2012. In the same period, trees gradually replaced matured leaves with old leaves, and leafless in March 13, 2012. It was noted that PCMA was entering the dry season when the total amount of precipitation was low. PCMA restarted radial growth when trees covered with leaves from May 8, 2012 and received much amount of precipitation. Radial growth and leaf phenology of PCMA, as well as total precipitation, were

related each other; radial growth slowed down when leaf shedding occurred during the period with less precipitation.

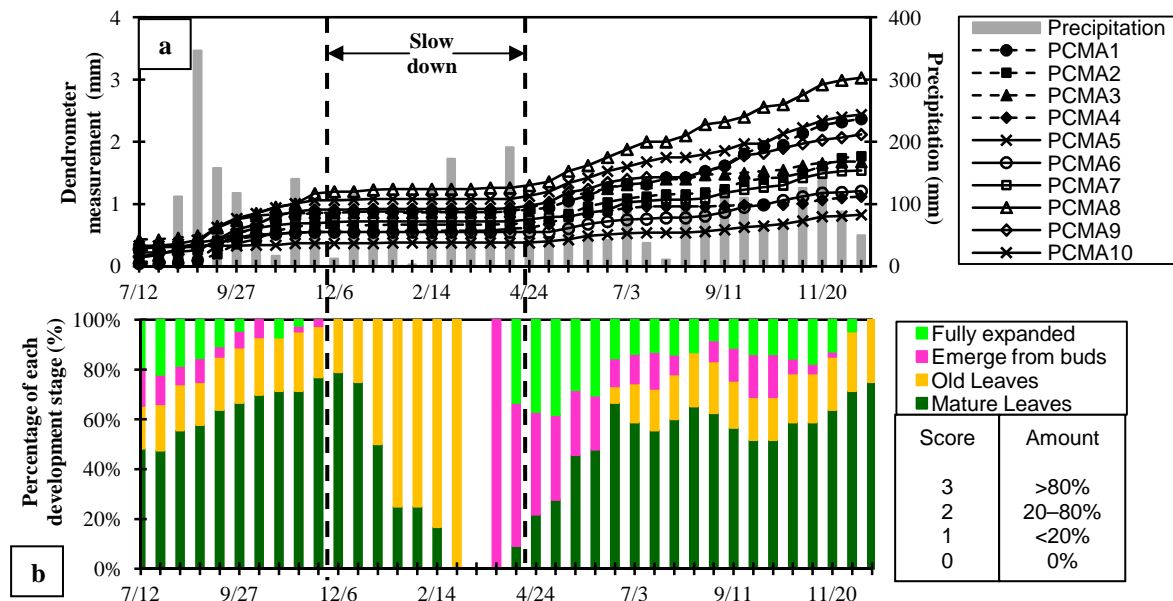


Fig. 4.2 Dendrometer measurement and precipitation (**a**) and leaf phenology (**b**) of *Peronema canescens* grown in Mata Ayer Forest Reserve. Radial growth and phenological observation were measured and observed every two weeks from July 12, 2011 until December 18, 2012. Two-week precipitation data was sorted after obtained from the Malaysian Meteorological Department.

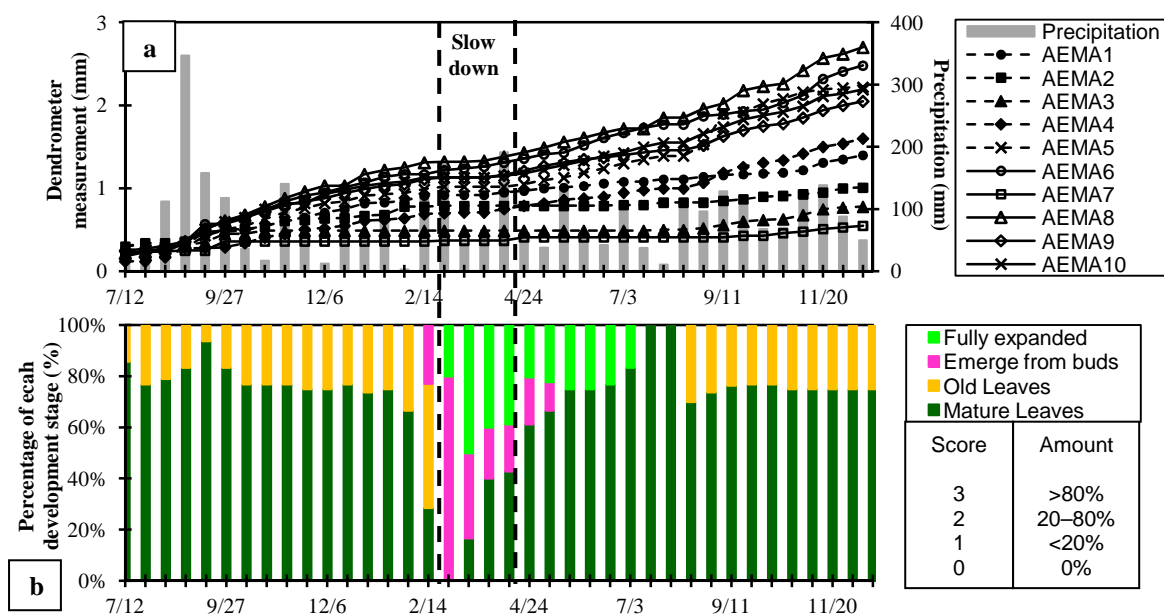


Fig. 4.3 Dendrometer measurement and precipitation (**a**) and leaf phenology (**b**) of *Azadirachta excelsa* grown in Mata Ayer Forest Reserve. Radial growth and phenological observation were measured and observed every two weeks from July 12, 2011 until December 18, 2012. Two-week precipitation data was sorted after obtained from the Malaysian Meteorological Department.

Azadirachta excelsa in MAFR (AEMA), in general, showed continuous radial growth (Fig. 4.3a), and did not show any particular period of leaf shedding; they always produced new leaves with keeping of old leaves (Fig. 4.3b). However, AEMA did showed slow growth in the early stage of

measurement until August 23, 2011. During this period, trees received less precipitation, but were covered with leaves. Radial growth restarted from September 13, 2011 and continued until February 14, 2012. In the same period, at least 30% of old leaves were replaced with new ones. Radial growth slowed down for a short period in the dry season from February, 28 to March 27, 2012. AEMA restarted radial growth when trees were covered with new leaves emerged from buds from April 10, 2012 and received much amount of precipitation. Radial growth of AEMA appeared to have responded with the dry period.

Hopea odorata in MAFR (HOMA), in general, also showed continuous radial growth (Fig. 4.4a), and did not show any particular periods of leaf shedding; they always produced new leaves with keeping of old leaves (Fig. 4.4b). Similar to AEMA, HOMA did show slow growth in the early stage of measurement until August 23, 2011. During this period, trees were covered with leaves, but received less precipitation. Radial growth restarted from September 13, 2011 until March 1, 2012. In the same period, trees were replaced with old leaves by at least 20%. Slowing down of radial growth occurred for a short period in the dry season from February 14 to March 27, 2012. HOMA restarted radial growth when trees received much amount of water. Radial growth of HOMA seemed to be responded with the water shortage when the radial growth slowed down during the dry period.

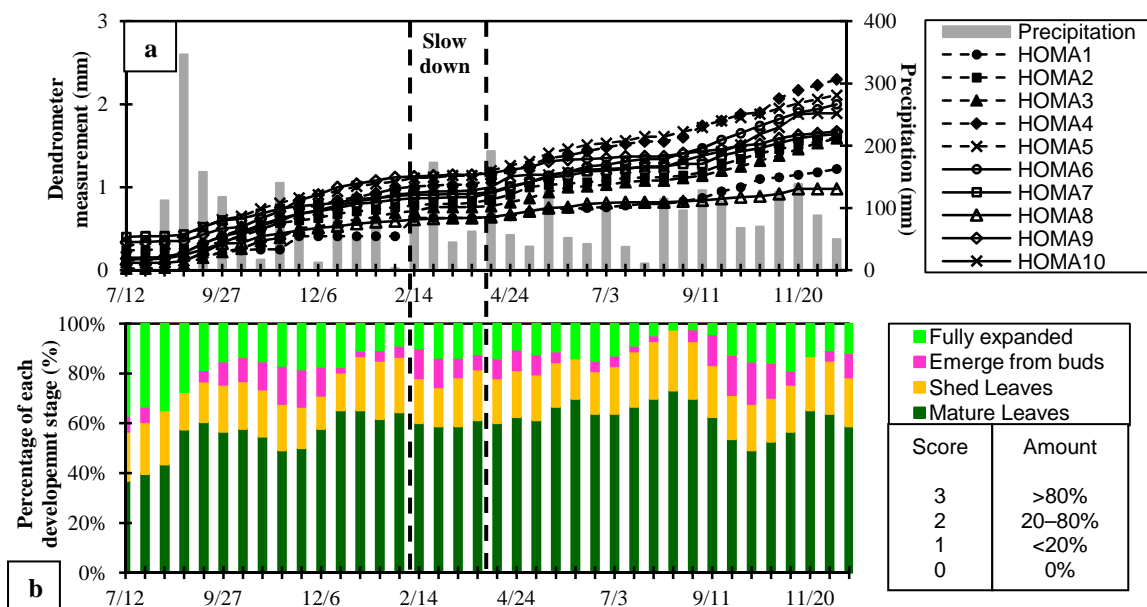


Fig. 4.4 Dendrometer measurement and precipitation (a) and leaf phenology (b) of *Hopea odorata* grown in Mata Ayer Forest Reserve. Radial growth and phenological observation were measured and observed every two weeks from July 12, 2011 until December 18, 2012. Two-week precipitation data was sorted after obtained from the Malaysian Meteorological Department.

4.3.2 Radial growth, impedance and leaf phenology of trees grown in BHFRIM

P. canescens in BHFRIM (PCBH) showed seasonal rhythm of radial growth and cambial activity (Fig. 4.5a and b). PCBH did not show any particular periods of leaf shedding; they always produced new leaves with keeping some amount of old leaf (Fig. 4.5c).

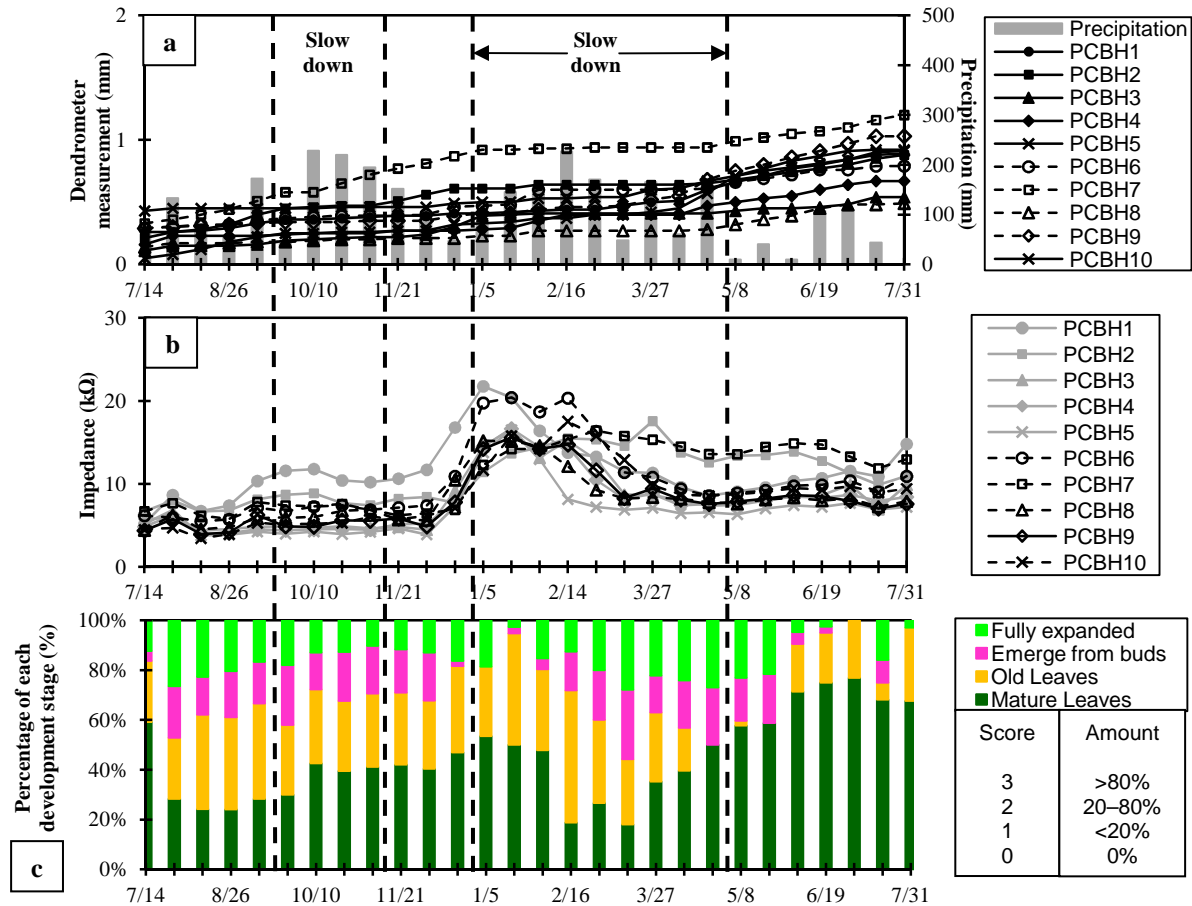


Fig. 4.5 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Peronema canescens* grown in Bukit Hari FRIM. Radial growth, impedance and phenological observation were measured every two weeks from July 14, 2011 until July 31, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

PCBH showed active growth in the early stage of measurement. During this period, impedance values were low. Some of the individuals showed first slowing down of radial growth from September 26, 2011 and continued until November 8, 2011. During this period, the impedance values slightly increased and trees were covered with old leaves by at least 20%. Radial growth was slowing down for the second time from January 5, 2012 and continued until April 24, 2012. In the same period, impedance values increased rapidly and then gradually decreased. When the impedance values reached the maximum, trees were covered with leaves by at least 50% and some of them were shed thereafter. When the impedance values gradually decreased, trees produced new leaves. PCBH

restarted radial growth when trees were covered with leaves and cambium was reactivated. Radial growth, impedance values and leaf phenology of PCBH were related to one another. Radial growth slowed down when the impedance values were high and leaf shedding occurred. Slowing down of radial growth in PCBH seemed to be not affected by much amount of precipitation. It was also showed that PCBH restarted and continued the radial growth during the low precipitation period. Based on the observation of site condition and tree stem, PCBH were planted close to a swamp and most of PCBH were covered with mosses. These conditions indicate that PCBH was grown in an extreme wet environment.

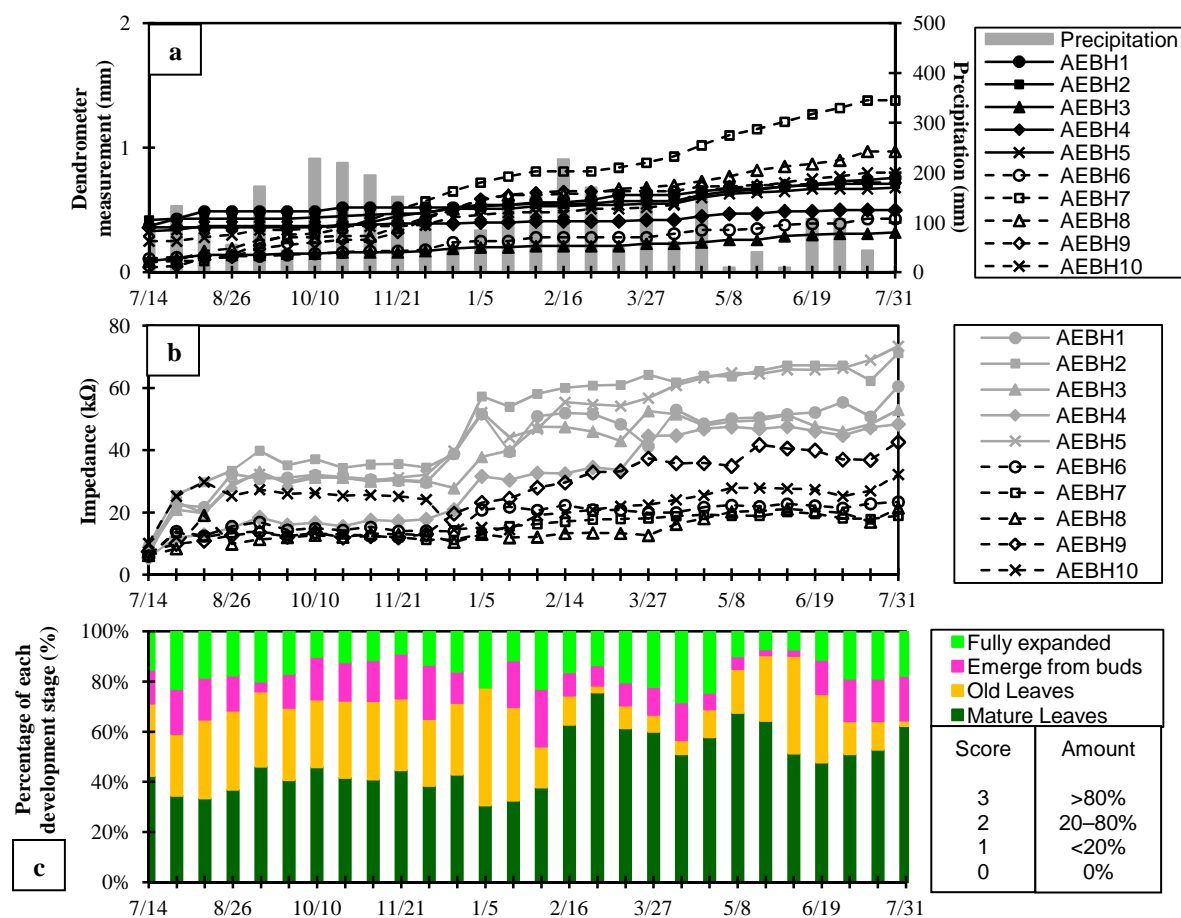


Fig. 4.6 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Azadirachta excelsa* grown in Bukit Hari FRIM. Radial growth, impedance and phenological observation were measured every two weeks from July 14, 2011 until July 31, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

A. excelsa in BHFRIM (AEBH) showed slow radial growth throughout the experimental period (Fig. 4.6a). All slow growing individuals always showed high impedance values (Fig.4.6b). AEBH had at least 30% old leaves from July 14, 2011 until January 19, 2012 (Fig. 4.6c). One or two

individuals showed slowing down of radial growth. Slowing down of radial growth was noticed for AEBH7 from January 31 to February 27, 2012, and the impedance values increased during this period.

H. odorata in BHFRIM (HOBH) showed continuous radial growth until the end of experimental period (Fig. 4.7a). The impedance values varied from low to high, depending on individuals (Fig. 4.7b). The evergreen HOBH were always covered with mature leaves and new leaves even there were also old leaves on the tree crowns (Fig. 4.7c). However, slowing down of radial growth in a short period was noticed from January 5 to January 19, 2012. During this period, impedance values were relatively low and no obvious leaf shedding occurred.

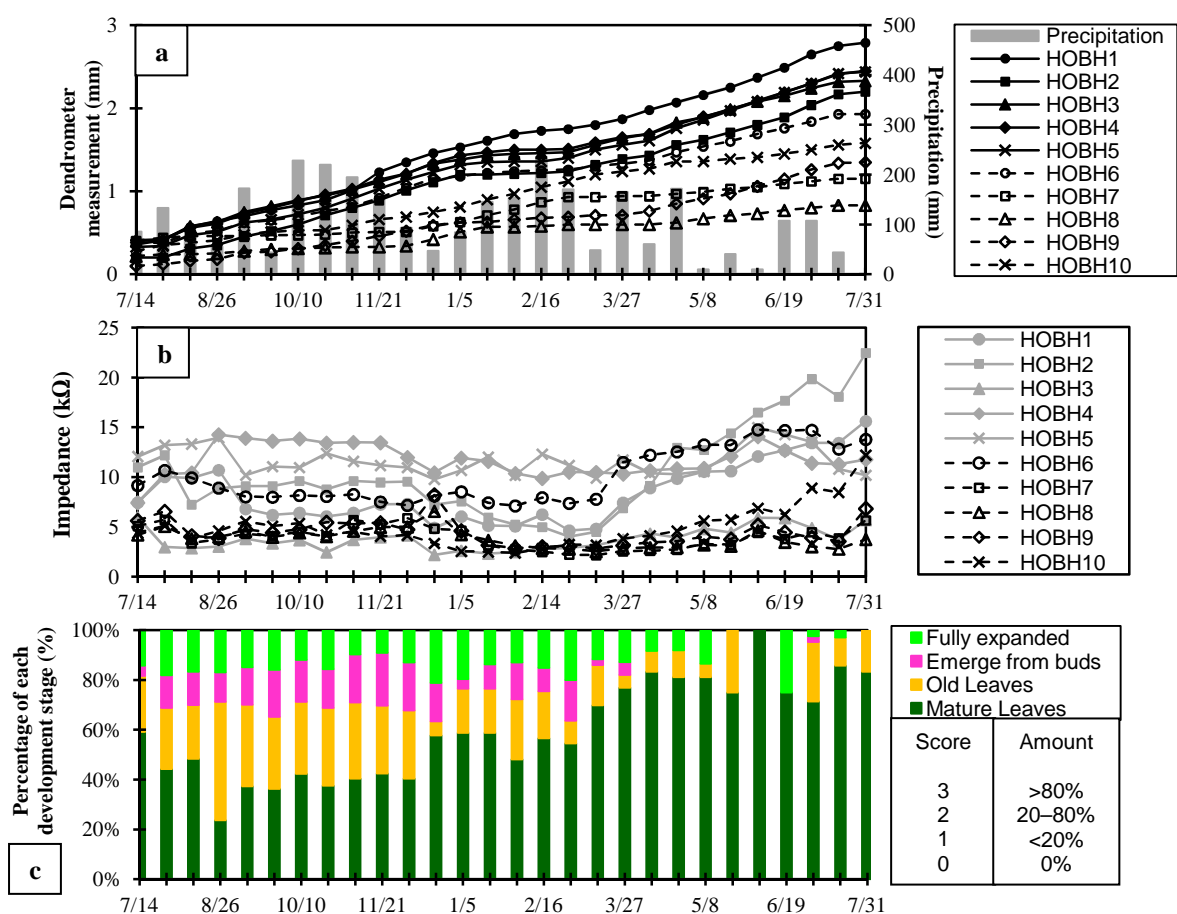


Fig. 4.7 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Hopea odorata* grown in Bukit Hari FRIM. Radial growth, impedance and phenological observation were measured every two weeks from July 14, 2011 until July 31, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

4.3.3 Radial growth, impedance and leaf phenology of trees grown in AHFR

Intsia palembanica in AHFR (IPAH) showed seasonal rhythm of radial growth and cambial activity (Fig. 4.8a and b). IPAH shedding leaves until leafless during the period of less precipitation (Fig. 4.8c). IPAH showed active growth in the early stage of measurement. During this period,

impedance values were low. Radial growth slowed down from April 24 until July 31, 2012. During this period, the impedance values slightly increased and trees were covered mostly with old leaves, and finally became leafless in July 17–31, 2012. AHFR received less amount of precipitation during this period. Radial growth restarted again, but in a slow pace. During this period, impedance values reached maximum and remained high until the end of experimental period. Radial growth, impedance values and leaf phenology monitored in IPAH were seemed to be related each other; radial growth slowed down when the impedance values gradually increased and leaf shedding continued until leafless during the less precipitation period.

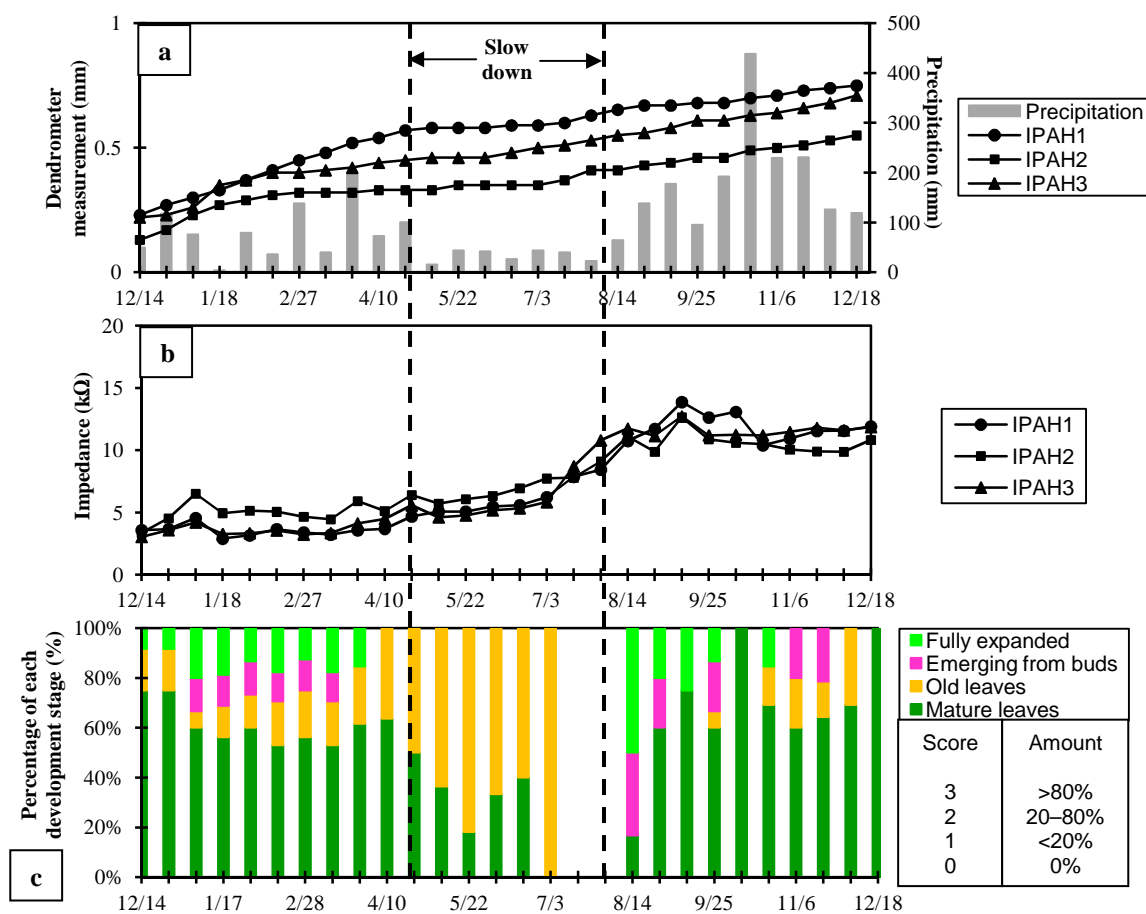


Fig. 4.8 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Intsia palembanica* grown in Ayer Hitam Forest Reserve. Radial growth, impedance and phenological observation were measured every two weeks from December 14, 2011 until December 18, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

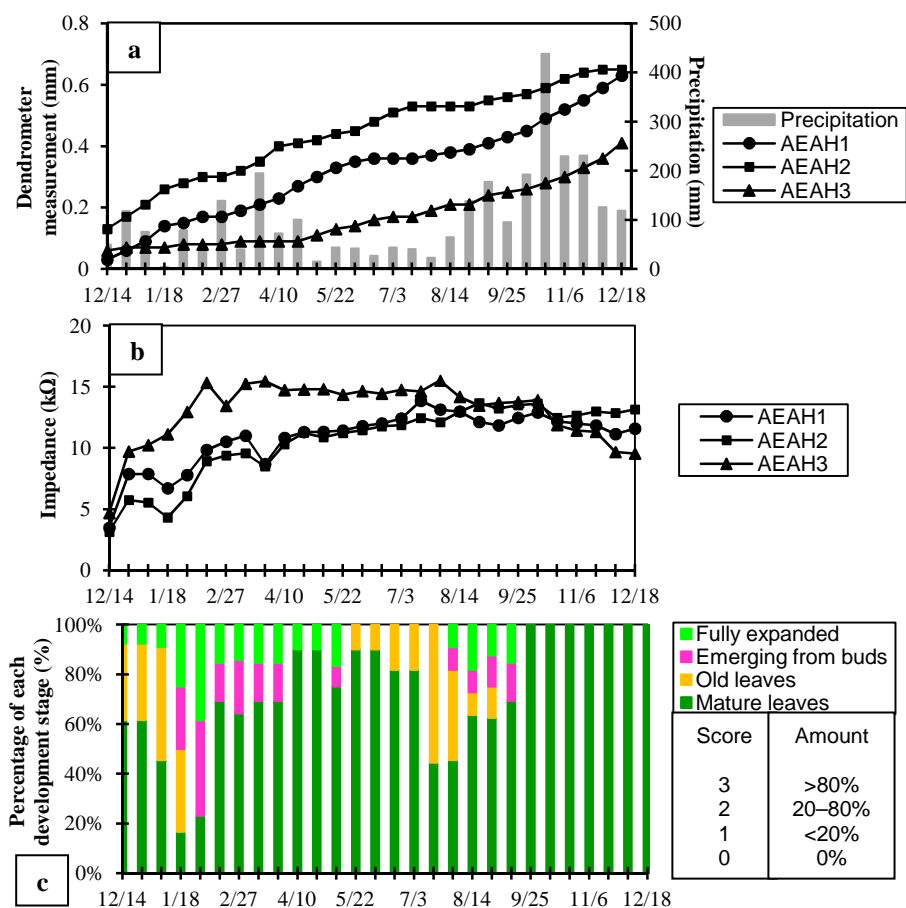


Fig. 4.9 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Azadirachta excelsa* grown in Ayer Hitam Forest Reserve. Radial growth, impedance and phenological observation were measured every two weeks from December 14, 2011 until December 18, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

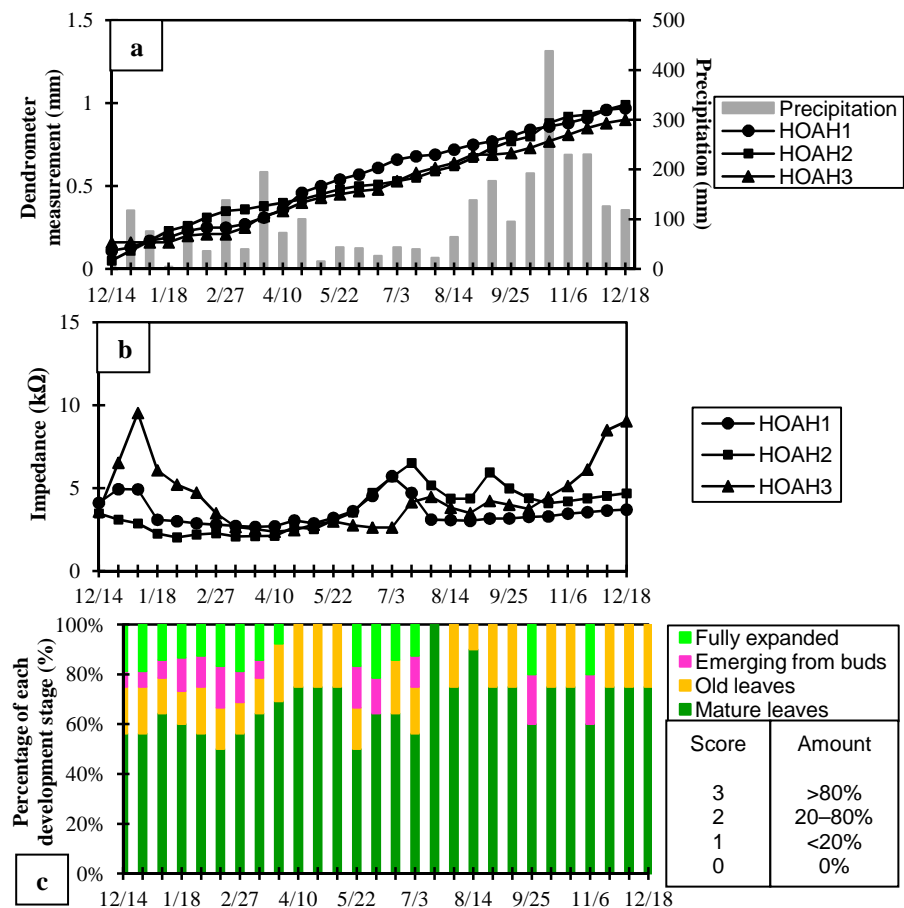


Fig. 4.10 Dendrometer measurement and precipitation (a), impedance (b) and leaf phenology (c) of *Hopea odorata* grown in Ayer Hitam Forest Reserve. Radial growth, impedance and phenological observation were measured every two weeks from December 14, 2011 until December 18, 2012. Precipitation data was obtained from the Malaysian Meteorological Department.

A. excelsa in AHFR (AEAH) showed continuous radial growth (Fig. 4.9a). The impedance values were high from the beginning until the end of experimental period (Fig. 4.9b). No particular shedding of leaves occurred in AEAH (Fig. 4.9c). However, there was a difference among individuals. AEAH1 and AEAH2 showed more faster radial growth based on dendrometer measurement and lower impedance values compared to AEAH3.

H. odorata in AHFR (HOAH) showed continuous radial growth and active cambial activity (Fig. 4.10a and b) since the beginning of the experimental period. Impedance values increased during the period of less precipitation and decreased during the period of much precipitation. HOAH was always covered with leaves and produced new ones (Fig. 4.10c).

4.4 Discussion

Seasonal rhythm of radial growth was detected for species with well-defined growth rings, *Peronema canescens* grown in MAFR (PCMA) and BHFRIM (PCBH), and *Intsia palembanica* in AHFR (IPAH). The changes in radial growth seemed to have synchronized with changes in precipitation (PCMA and IPAH), leaf phenology (PCMA and IPAH), and cambial activity (PCBH and IPAH). It was considered that each factor were independent of the features in the visible growth rings in *P. canescens* and *I. palembanica*. However, it cannot be concluded yet because there are other stimulus factors involved in growth ring formation.

One of the external factors closely associated with leaf fall in the tropics is drought. Medway (1972) reported that leaf fall behavior in the tropics is correlated with the occurrence of dry period, even if it is short and unpredictably throughout a year. Alvim (1964) reported that in the extensive area of north-eastern Brazil which covered by the thorn-deciduous forest, all trees lose their foliage during the dry season and produce new leaves shortly after the onset of rains. In deciduous trees, leaf shedding almost invariably occurs during the dry period. This influence of moisture deficiency has been noticed by Koriba (1958), even in areas with uniform rainfall distribution, where several species lose their leaves after relatively short dry spells, sometimes twice or even three times during the year. In the periodically dry region of eastern Java, teak (*Tectona grandis*) loses its leaves during the dry

season, but when cultivated in the wetter area of western Java, it behaves like an evergreen (Alvim, 1964).

The relationship between leaf changes and cambial activity was studied by Koriba (1958) and Alvim (1964). During the leafless period of deciduous species, the cambium does not divide and activity is not resumed until the new leaves unfold. This cessation of cambial activity makes the rings quite visible. Alvim (1964) stated that clear rings in tropical regions are found only in deciduous trees, but Chowdhury (1940a) found well-marked rings in some tropical evergreens, such as *Pterocarpus dalbergioides*, *Colubrina* sp., and *Peronema* sp.

Weak seasonal rhythms of radial growth were detected in poorly-defined growth ring *Azadirachta excelsa* grown in MAFR (AEMA) and indistinct growth ring of *Hopea odorata* in MAFR (HOMA) and BHFRIM (HOBH). In general, they seemed to grow continuously. The growth habit was termed as weakly seasonal because the changes in radial growth seemed to respond to the changes in precipitation. They shed old leaves with producing new leaves at the same time. No variations in cambial activity were detected based on impedance measurement.

A common feature for trees in the humid tropics is the synchronous occurrence of leaf flush and leaf fall. For this semi-deciduous nature, the term leaf emergence or leaf change has been coined (Longman and Jenik, 1974). Characteristically these trees are never truly deciduous, but always carry old and young leaves at the same time. The relative importance of the external and internal factors in controlling leaf fall in tropical climates has been much discussed (Richards, 1957, Koslowzki, 1971a, Kramer and Kozlowski 1979). In evergreen trees, leaf fall generally follows the rhythm of leaf production, and the phenomenon seems to suggest a competition for carbohydrates and/or plant hormones or nutrients between young and old leaves. In such cases, an internal mechanism connected with leaf abscission is probably present, although its primary cause derives from factors including leaf production.

Slow growth and high impedance values in most of *A. excelsa* in BHFRIM (AEBH) individuals seemed not affected by less precipitation or changes in leaf phenology. One of the possible reasons was that AEBH were planted on a hill slope. Based on the observation of site condition and tree morphology, some other stands of *A. excelsa* were affected with die-back disease.

It might happen that selected experimental trees were also affected by die-back. In AHFR, one of the possible reasons of different growth rate among *A. excelsa* trees was that AEAH1 and AEAH2 were grown in an open condition with no competition with other trees, while AEAH3 were grown in a closed environment under other emergence trees.

4.5 Summary

In this study, it was shown that some of tropical rainforest trees grown under tropical monsoon and rainforest climate had seasonal rhythm in radial growth, synchronized with changes in precipitation and leaf phenology as well as cambial activity. Well defined growth ring of *Peronema canescens* grown in MAFR showed seasonal radial growth when the radial growth slowed down and shed of leaves during low precipitation (dry period). *P. canescens* in BHFRIM also showed seasonal radial growth when radial growth slowed down in a certain period. It was considered that it did not respond to the changes in precipitation or leaf phenology, but stressed by humid condition. *Intsia palembanica* grown in AHFR showed seasonal rhythm of radial growth and cambial activity. Slowing down of radial growth and inactive cambial activity were noticed during less precipitation and shedding of leaves. The clarification of growth ring formation during those events will be discussed in the next chapter.

Chapter 5

Characterization of growth ring formation in tropical rainforest trees

5.1 Introduction

Chapter 1 discussed the possibility to investigate the wood formation in tropical rainforest trees. Important points on the occurrence and distinctiveness of growth ring features were discussed in Chapter 2. Chapter 3 discussed the effectiveness of cambial marking and stable carbon isotope to study wood formation in tropical rainforest trees. Radial growth, cambial activity, and leaf phenology of the species groups having well-defined, poorly-defined and absent growth rings have been discussed in Chapter 4. Aiming to the main goal, detection of annual ring formation was investigated in the species group of well-defined growth rings. It is important for promoting tropical dendrochronology to clarify and confirm whether those well-defined growth rings are formed annually or not.

Investigation of the growth ring and clarification of its annual formation in present study was approached by anatomical and isotopic analysis, coupled with results obtained through dendrometer measurement, phenological observation, impedance measurement, and precipitation record.

5.2 Materials and Methods

5.2.1 Samples

Samples selected for this study belonged to the group of well defined growth rings. The sample species used were *Peronema canescens* grown in MAFR (PCMA9) and BHFRIM (PCCP10), and *Intsia palembanica* grown in AHFR (IPAH1).

Wood blocks contained DC-pulse marks made in July 2011 (for samples in MAFR and BHFRIM) and December 2011 (samples in AHFR) were collected in January 2013. Wood blocks from MAFR and BHFRIM included 19-month of radial growth information while those from AHFR included that of 12-month. December-marked position was also included in the July-marked samples.

5.2.2 Anatomical observation and stable carbon isotope analysis

The position of DC-influenced cells in the wood samples were identified macroscopically and microscopically. The identified marked positions were referred to to detect the occurrence of growth ring feature along the radial strip and to prepare the samples for isotopic analysis. Anatomical features of growth rings and irregularities discovered in Chapter 2 were examined.

The results of stable carbon isotope and macroscopically distinct growth ring features were combined with individual data of radial growth, leaf phenology and electrical impedance of studied samples. The distinctiveness of annual rings was examined based on the synchronicity of anatomical changes, variations of $\delta^{13}\text{C}$ values, rhythm of radial growth, leaf phenology, and precipitation during a known period.

5.3 Results

5.3.1 Characterization of growth ring formation

Annual variations of $\delta^{13}\text{C}$ values, occurrence of growth rings, diameter growth, precipitation, and leaf phenology of *Peronema canescens* grown in MAFR (PCMA9), BHFRIM (PCBH10) and *Intsia palembanica* in AHFR (IPAHI) were shown in Figs. 5.1, 5.2 and 5.3.

Markings in PCMA9 were first applied in July 12, 2011 and December 25, 2011 for the second time. Based on macroscopic observation, one growth ring boundary was formed within a 19-months period of experimental period (Fig. 5.1b). Focusing on the position between previous and current growth ring boundaries (dashed lines), the values of $\delta^{13}\text{C}$ varies with the anatomical changes in PCMA9, indicated by Zones 1, 2 & 3 (Fig. 5.1a). In Zone 1, the values of $\delta^{13}\text{C}$ rapidly increased to the maximum. Anatomically, it was featured by large vessels. A possible reason of increased values of $\delta^{13}\text{C}$ in Zone 1 was considered as a result of the use of some carbon reserves when entering the initial stage of new xylem formation. In Zone 2, the $\delta^{13}\text{C}$ values gradually decreased to the minimum. Anatomically, it was featured by large vessels. In Zone 3, the $\delta^{13}\text{C}$ increased to the maximum when the vessels change to smaller sizes. In the region between the end of Zone 3 to the position of marking in July 2011, the $\delta^{13}\text{C}$ values gradually decreased. Based on the values of $\delta^{13}\text{C}$ and anatomical features from the start of previous ring boundary to the marking position, PCMA9 did show a cyclic

variations in $\delta^{13}\text{C}$ values and vessel sizes. Therefore, the cyclicity pattern of $\delta^{13}\text{C}$ values and changes of vessels size were referred to evaluate the occurrence of growth ring in current study.

The positions of July and December marking (9.7 mm and 6.3 mm from cambium) were located in the region of previous growth ring. From the position of July marking, the values of $\delta^{13}\text{C}$ continued decrease to the minimum from the previous peak at the end of Zone 3. Anatomically, this region was featured by small vessels (Zone 4). PCMA9 showed continuous radial growth between the two marking periods, during which the amount of precipitation was high (Fig. 5.1c) and the tree was covered with leaves (Fig. 5.1d).

From the position of December marking to the end of Zone 5, the values of $\delta^{13}\text{C}$ gradually increased and reached a maximum, and decreased until the end of the period. Large vessels were observed in the beginning of Zone 5 and changed to small vessels. It was believed that during the December marking, a new growth ring was formed in PCMA9. Increase in $\delta^{13}\text{C}$ values during the formation of new large vessels in Zone 5 corresponded with the result of Zone 1. The radial growth of PCMA9 slowed down and old leaves were lost and became leafless in March 13–27. Increase of $\delta^{13}\text{C}$ values in the pore zones in PCMA9 was attributable to the use of some carbon reserves at the initial stage of new xylem formation. The maximum value of $\delta^{13}\text{C}$ was located at the position of medium-size vessels in the middle of Zone 5. It might be the point where PCMA9 started to reactivate radial growth. Values of $\delta^{13}\text{C}$ gradually decreased until the end of the experimental period. Anatomically, it was featured by gradual changes from medium to small size vessels. The radial growth continued until the end of the period when precipitation increased and tree was covered with leaves.

It is considered that the new ring boundary in PCMA9 were formed either in late December 2011 or in early January 2012, during which precipitation was low and leaf shedding initiated.

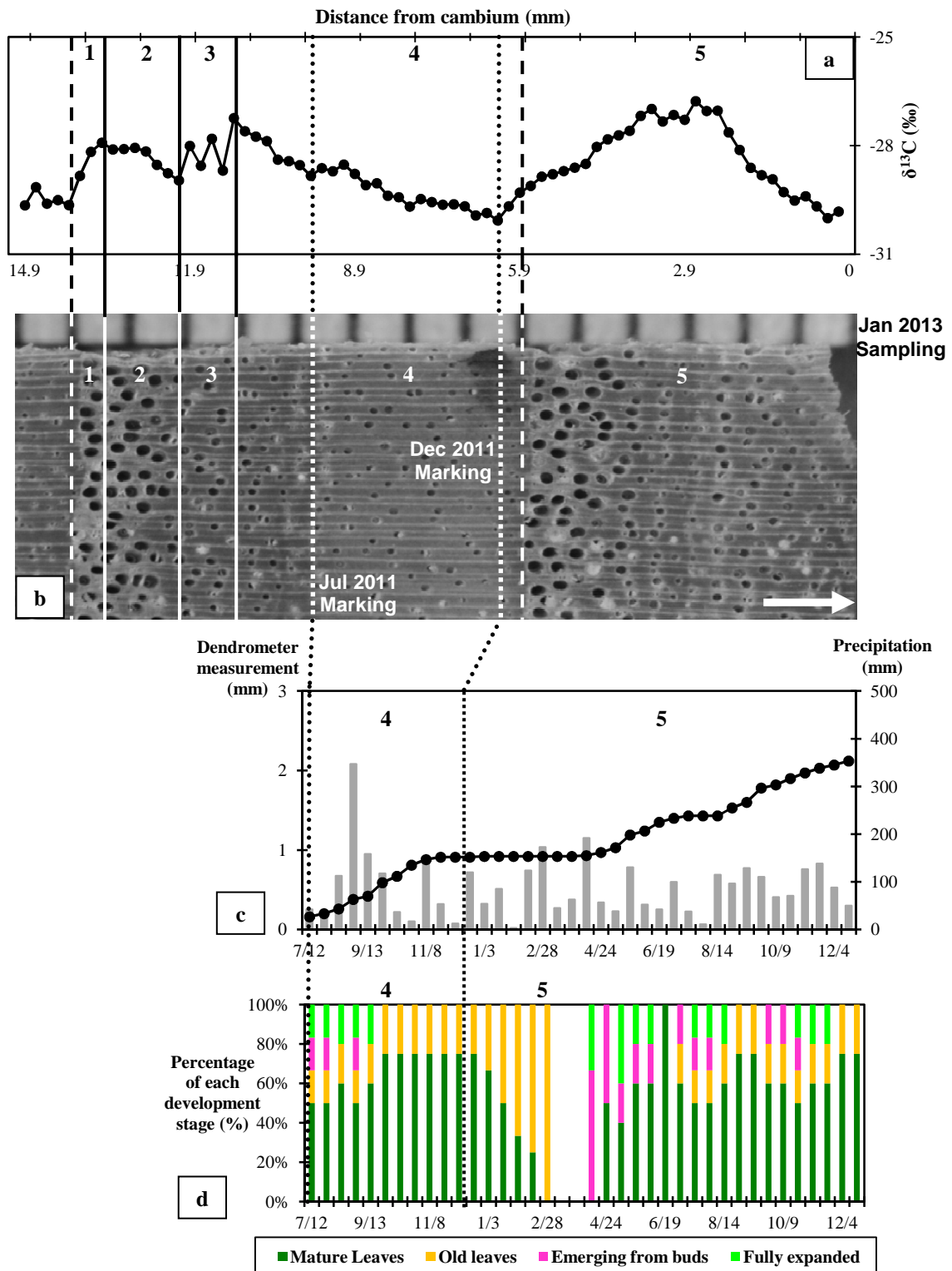


Fig. 5.1 Variations of $\delta^{13}\text{C}$ (a), transverse surface (b), dendrometer measurement and precipitation (c), and leaf phenology (d) of *Peronema canescens* (PCMA9) grown in Mata Ayer Forest Reserve. White arrow in (b) indicates the direction of the bark side. Dotted lines indicate marking position, made on July 12 and December 25, 2011. Dashed lines indicate the growth ring boundaries. Solid lines demarc different growth stages.

Markings in PCBH10 were first applied in July 14, 2011 and December 28, 2011 for the second time. Unlike PCMA9, PCBH10 features the characteristics of diffuse-porous wood. Therefore, they limit the use of vessel size differences as the growth indicator. One of the possible reasons of diffuse-porous features might be that PCBH10 grew close to a swamp in BHFIRIM. Based on the anatomical observation, one growth ring boundary was considered to have been formed within 19-months of the experimental period, evidenced in changes of vessel density and/or size (Fig. 5.2b).

In Zone 1, the values of $\delta^{13}\text{C}$ increased to the maximum and gradually decreased (Fig. 5.2b). Anatomically, the size of vessels was slightly larger and the density was higher. In Zone 2 where the July's marking was located, the values of $\delta^{13}\text{C}$ decreased to the minimum and increased to the maximum. In this region, vessels gradually changed to smaller sizes and became low in density. Based on the dendrometer measurement, radial growth was considered not to have reactivated since the beginning (Fig. 5.2c), but cambium may have shown some activity based on low impedance values (Fig. 5.2d). During this period, the amount of precipitation was high and tree was always covered with leaves, even shedding of leaves occurred (Fig. 5.2e).

In the region between December's marking and estimated ring boundary (Zone 3), the values of $\delta^{13}\text{C}$ decreased. Anatomically, it was featured by low vessel density and small vessel sizes. The ground tissue was darker, featured by thick-walled fibers microscopically. During this period, the radial growth was slowly reactivated with the increasing amount of precipitation while the tree was covered with leaves. High impedance values were recorded during this period, indicating that cambium had a low activity. It was considered that during this period, PCBH10 was forming wood fibers rather than vessels so as to increase the mechanical strength in stem.

In Zone 4, where the estimated region of ring boundary was formed, the values of $\delta^{13}\text{C}$ always fluctuated and did not show any obvious trends. Anatomically, there was a change in vessel density along the radial direction, but it did not correspond to the variation of $\delta^{13}\text{C}$ values. Radial growth was estimated to be reactivated and the cambial activity was low until the end of the period. In this study, it was considered that the selected stems of PCBH10 should be classified as having anatomically absent growth rings. Based on the $\delta^{13}\text{C}$ values, PCBH10 shows no cyclic variations. It

was considered that clear isotopic cyclicity of PCBH10 might be observed in stable oxygen isotope ($\delta^{18}\text{O}$) analysis, since PCBH10 was growing under an extremely wet condition.

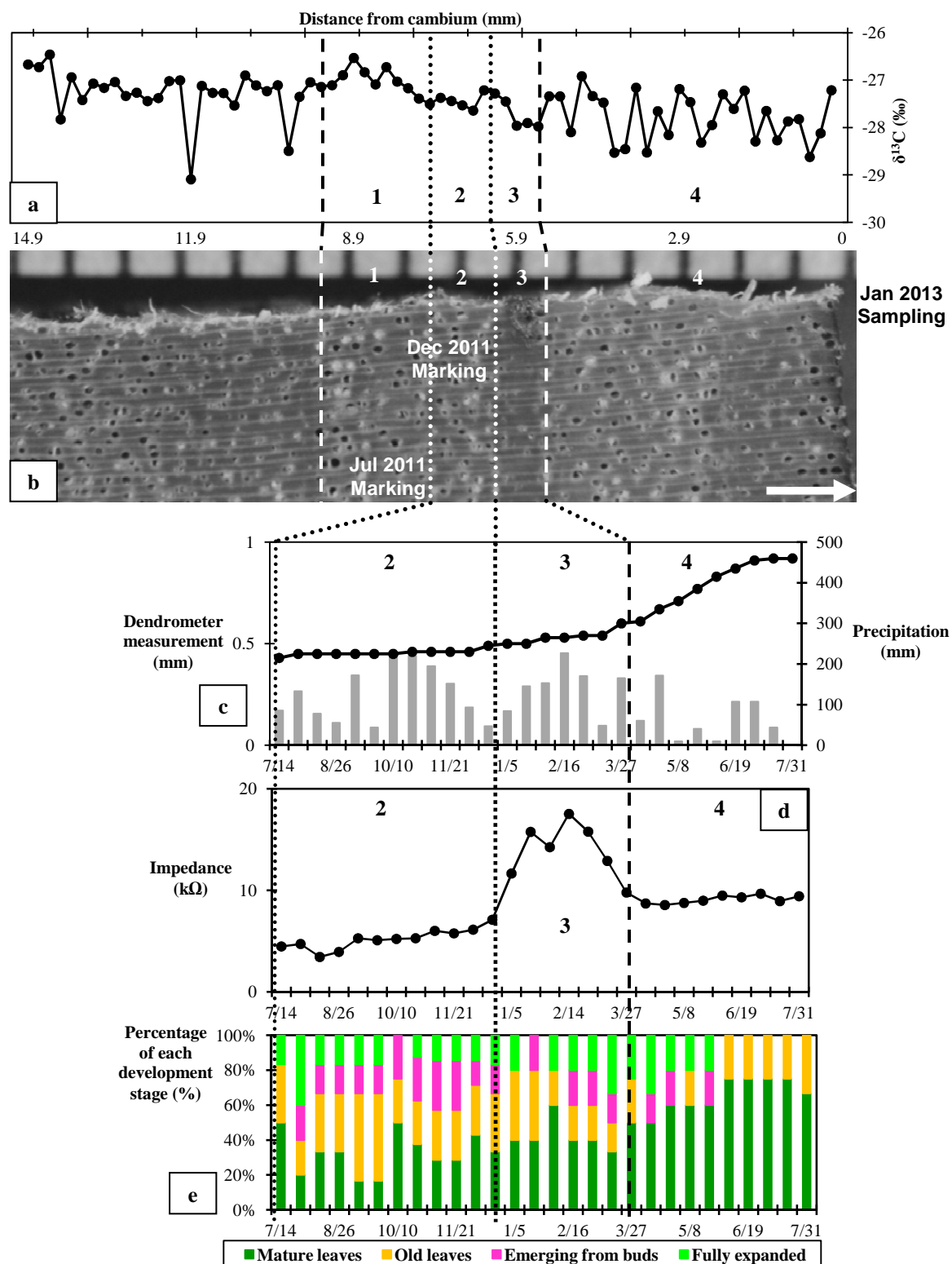


Fig. 5.2 Variations of $\delta^{13}\text{C}$ (a), transverse surface (b), dendrometer measurement and precipitation (c), impedance (d), and leaf phenology (e) of *Peronema canescens* (PCBH10) grown in Bukit Hari FRIM. White arrow in (b) indicates the direction of the bark side. Dotted lines indicate marking position, made on July 14 and December 28, 2011. Dashed lines indicate the growth ring boundaries.

Marking in IPAHI was applied in December 27, 2011. Unlike *P. canescens*, IPAHI possesses the characteristics of diffuse-porous wood, thus it limits the use of vessel size differences as the growth indicator. Based on anatomical observation, one growth ring boundary was considered to be formed within a 12-months of experimental period, featured by initial or terminal parenchyma, i.e. marginal parenchyma (Fig. 5.3b).

Before the marking was applied, some ring boundaries were observed (Zone 1–6). IPAHI was considered not showing any variations in $\delta^{13}\text{C}$ values along the radial strip (Fig. 5.3a). However, it was shown that the position of terminal parenchyma in IPAHI had or was approaching the maximum value of $\delta^{13}\text{C}$. In Zones 1, 2 and 5, the maximum values of $\delta^{13}\text{C}$ were indicated by the position of terminal parenchyma. Anatomically, the terminal parenchyma was seemed to have occurred after or just after small vessel formation, before larger vessels were formed. The decreased values of $\delta^{13}\text{C}$ were indicated by the slightly large vessels. It was considered that the sharp peak arose from small vessels and the decreased values of $\delta^{13}\text{C}$ afterwards were the signals where IPAHI continue wood formation, judging from the appearance of the large vessels. It was considered that the formation of each terminal parenchyma in those zones were affected by either low moisture availability or shedding of leaves. In Zones 3 and 4, the parenchyma band was considered to be the initial parenchyma. Anatomically, this parenchyma band was associated with or connected among the large vessels. The parenchyma band was obtained between the minimum and maximum $\delta^{13}\text{C}$ values. It might be a signal indicating that IPAHI was reserving carbohydrates before entering the beginning or the end of the growth.

When the marking was applied in December 2011, the values of $\delta^{13}\text{C}$ slightly decreased. Anatomically, it was the position of large vessels. At this point, radial growth was continuous (Fig. 5.3c), high cambial activity (Fig. 5.3d) and tree was covered with leaves (Fig. 5.3e). $\delta^{13}\text{C}$ values were then increased to the maximum and they gradually decreased until the end of the period.

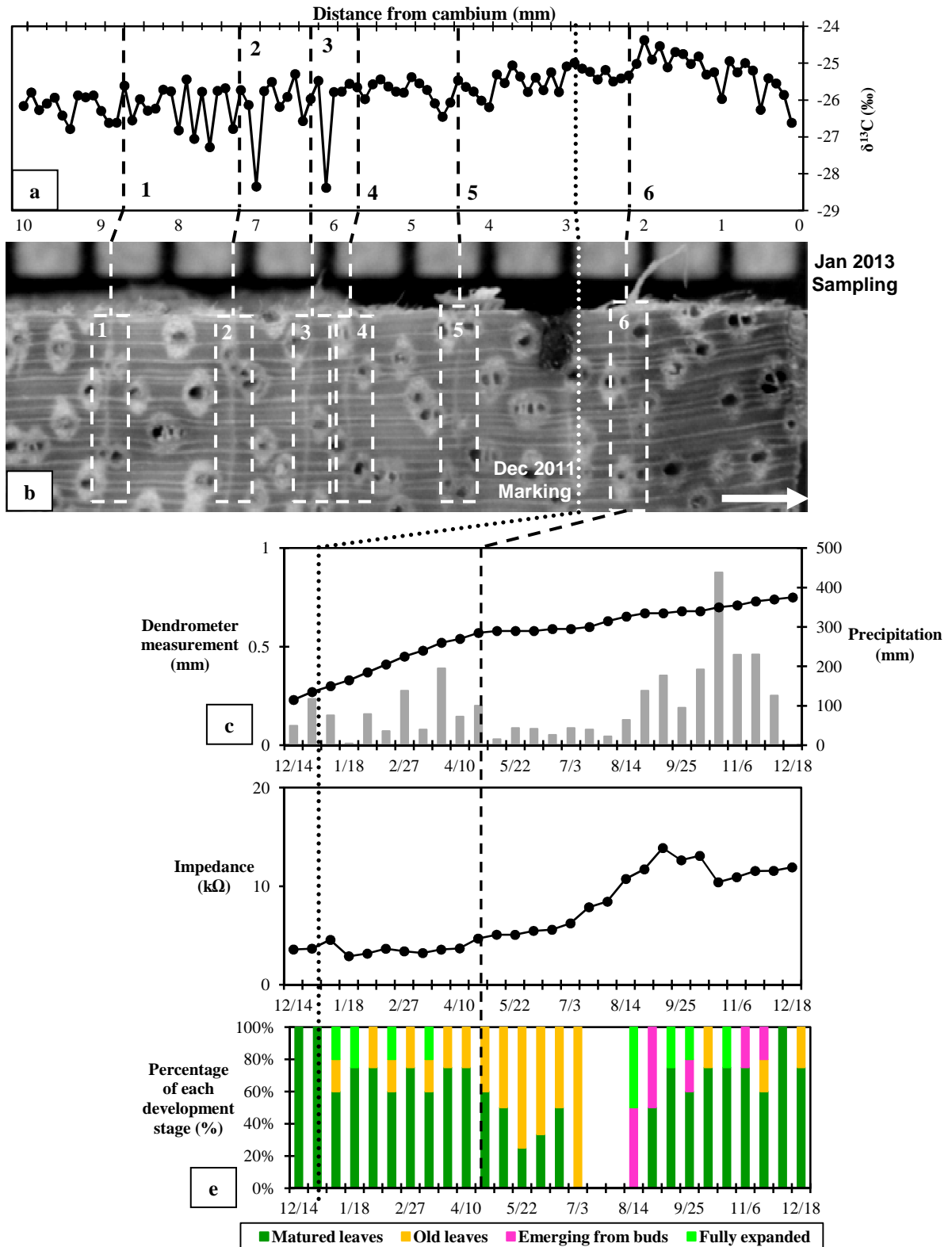


Fig. 5.3 Variations of $\delta^{13}\text{C}$ (a), transverse surface (b), dendrometer measurement and precipitation (c), impedance (d), and leaf phenology (e) of *Intsia palembanica* (IPAH1) grown in Ayer Hitam Forest Reserve. White arrow in (b) indicates the direction of the bark side. Dotted lines indicate marking position, made on December 27, 2011. Dashed lines and dashed squares indicate the growth ring boundaries.

Ring boundary was observed to have appeared after the marking (Zone 6). Zone 6 represents the values in between low and high $\delta^{13}\text{C}$. Anatomically, it was the terminal parenchyma, judging from the association with small vessels before the appearances of large vessels. During this period, it was estimated that small vessels and terminal parenchyma were formed during the slowing down of radial growth (Fig. 5.3c), high to low cambial activity, leafless, and low amount of precipitation. The decreased values of $\delta^{13}\text{C}$ coincide with a region of large vessels. At this point and toward the cambium, the radial growth was considered to have restarted and slowly continued. During this period, the cambial activity was considered low even when the tree was flushing new leaves and received high precipitation. It was considered that IPA1 was focusing more on flushing leaves during the slow and continuous radial growth. The appearance of large vessels was judged to be the signal of high precipitation.

However, it was considered that the appearance of ring boundary in IPA1 in this study cannot be clarified as an annual ring, and the tree was judged as having poorly-defined growth rings. It was because the values of $\delta^{13}\text{C}$ in IPA1 did not clearly varied as in PCMA9 and the relationship between the feature of marginal parenchyma with $\delta^{13}\text{C}$ values is unknown. Even though the appearances of marginal parenchyma can be relate with seasonal storage of photosynthates, it doesn't mean that this feature are formed annually.

5.4 Discussion

Precise dendrochronological studies require trees with annually distinct growth rings. However, the amount of trees with potential for dendrochronological studies in the tropics were restricted, and only *Tectona grandis* so far, shows promise for dendrochronology (Pumijumnong 2013). In addition, tropical dendrochronology was mostly been applied to those trees grown under tropical seasonal climate and limited to tropical rainforest area. In this study, the author showed the potential of species belonging to well-defined growth ring, which were *Peronema canescens* grown in tropical monsoon and rainforest regions, and *Intsia palembanica* grown in tropical rainforest area.

The ring-porous feature in *P. canescens* was considered as genetic, enabling its use as a growth descriptor. Ring-porous feature of *P. canescens* in MAFR (PCMA9) allows the author to

distinguish the growth ring anatomically. Based on the cyclic variation of $\delta^{13}\text{C}$ values, it was considered that PCMA9 experienced seasonality in moisture availability when the $\delta^{13}\text{C}$ values increased during the dry period and decreased during the rainy period. The formation of large vessels during the rapid increased of $\delta^{13}\text{C}$ values were considered to be related to the use of reserve material when the tree shed the leaves during a dry period and slowed down the radial growth. Changes in anatomical features and cyclic variations of $\delta^{13}\text{C}$ values were estimated to be synchronized with the rhythms of radial growth, changes in precipitation and leaf phenology. Thus, the growth ring boundary formed within the experimental period was considered to be an annual ring and estimated to be formed between January and March 2012, during the dry period, slowing down of radial growth, and shedding of leaves.

Growth ring was difficult to be identified in the selected *P. canescens* in BHFRIM (PCBH10), because it was featuring diffuse-porous wood. One of the possible reasons that PCBH10 showed diffuse porosity that it grew close to a swamp and received much water. Based on the $\delta^{13}\text{C}$ values, it was shown that PCBH10 did not experience any difficulty in water supply. The author assumed that wet conditions restricted PCBH10 to show seasonal rhythm of radial growth and having changes in leaf phenology. However, information on environmental water sources and atmospheric vapor pressure deficit through an analysis of oxygen isotope ratio (Roden et al 2000), might enable us to interpret the variations of anatomical changes on an ecological basis and detect growth rings in PCBH10. It was considered that influence of site conditions should be emphasized for further study of growth ring formation in well-defined growth ring in *P. canescens* grown under the tropical rainforest climate. In this study, therefore, the author classified PCBH10 as having ambiguous to absent growth ring based on inability to integrate anatomical changes and values of $\delta^{13}\text{C}$ with precipitation, radial growth, cambial activity, and leaf phenology.

Growth rings of *I. palembanica* in AHFR (IPAHI) was demarcated by marginal parenchyma macroscopically and one growth ring occurred during the experimental period. Microscopically, IPAHI featuring the terminal and/or initial parenchyma, being judged by the shape of the cells. The former was mostly radially flattened in shape and associated with small vessels while the later mostly varied in shape and associated with large vessels. Based on the values of $\delta^{13}\text{C}$, IPAHI did not show

any cyclic variations, but the position of terminal parenchyma was noticed to have a high value of $\delta^{13}\text{C}$. It was unclear to relate nature of parenchyma and the values of $\delta^{13}\text{C}$, thus, the author considered that the high and low values of $\delta^{13}\text{C}$ was aroused from small and large vessels. Based on the occurrence of present parenchyma band, values of $\delta^{13}\text{C}$, rhythm of radial growth, cambial activity, leaf phenology, and precipitation, it was estimated that the terminal parenchyma was formed during the period of slowing down of radial growth, high to low cambial activity, shedding of leaves until becoming leafless, and low precipitation. Carlquist (2001) explained that growth rings featured by marginal parenchyma can be related to seasonal storage of starch and to flushes of growth. Dünisch and Puls (2003) explained the highest content of starch was found in the secondary xylem during a period of complete leafless when the cambium is dormant in dry period. At the locations of high value of $\delta^{13}\text{C}$, in which terminal parenchyma associated with small vessels is located, the values of $\delta^{13}\text{C}$ gradually decreased. Anatomically, this region was featured by large vessels. During this period, it was estimated that the radial growth restarted growth in slow mode when the tree was producing new leaves and received much water. Carlquist (2001) explained that the alternative possibility for the function of terminal parenchyma would be the storage of starch to support rapid flushes of growth, flowering, and fruiting. Dünisch and Puls (2003) found that wet condition decreased the amount of reserve materials, as for reactivation of cambial growth and formation of new leaves. In this study, it was considered that the terminal parenchyma formed during the experimental period was parallel with the availability of water and physiologically reacted to leafless period. However, the relationship between the occurrence of terminal parenchyma and variations of $\delta^{13}\text{C}$ values was considered weak even though they showed some relationship with rhythms of radial growth, cambial activity, leaf phenology and precipitation. Therefore, IPAH1 was considered and clarified as having a poorly-defined growth ring. The uncertainty of its annual formation can be investigated through the application of stable oxygen isotope analysis and analysis of changes in starch contents.

5.5 Summary

In this study, the characterization of annual rings was based on synchronicity of annual cyclic variations of $\delta^{13}\text{C}$ values, seasonal rhythm of radial growth pattern, cambial activity, changes in leaf phenology and precipitation in the well-defined group. With all combined parameters, PCMA9 featuring ring-porous wood was clarified as forming annual rings and the ring was considered to be formed during the period of slowing down of radial growth, leafless and less precipitation. PCBH10 featuring diffuse-porous wood was clarified as having ambiguous or no growth rings since they did not show any relationship between anatomical features and variations of $\delta^{13}\text{C}$ values with radial growth, cambial activity, leaf phenology, and precipitation. IPAH1 featuring marginal parenchyma was clarified as having poorly-defined growth rings because the marginal parenchyma was expected can be formed many times within a year, depending on how frequent the tree shed their leaves and experienced low precipitation. Detection of annual changes in PCBH10 and IPAH1 was considered possible by the analysis of stable oxygen isotope in the future.

Conclusions

The possibility of growth ring occurrence in tropical rainforest trees was shown in Chapter 1 and 2 through anatomical investigation, dendrometer measurement and leaf phenology. The anatomical features of growth rings were observed to be the fiber zones, radially-flattened fibers, thick-walled fibers, marginal parenchyma, and variations in vessel size and density. However, difficulties might be encountered: intermittent growth rings, ambiguous growth rings, discontinuous growth rings, and occurrence of traumatic resin canals in parenchyma bands. Based on the preliminary investigation, it was shown that *Peronema canescens* grown in tropical monsoon climate and rainforest climate area, and *Intsia palembanica* grown in tropical rainforest area had distinct and well-defined growth rings. 17 species showed poorly-defined growth ring and growth rings were absent in 10 species. Some of the tropical rainforest trees showed seasonal rhythm of radial growth and cambial activity when the radial growth slowed down during the period of shedding of leaves and low precipitation. During this period, the number of cambial and enlarging zone cells decreased. Therefore, Chapter 1 and 2 concluded that growth ring features occur in tropical rainforest trees and do show seasonal rhythm of radial growth and cambial activity.

Application of DC-pulse marking and stable carbon isotope analysis in Chapter 3 meet the expectation for precise investigation of wood formation in tropical rainforest trees. The DC-influenced cells such as crushed cells, crushed vessels, extended ray parenchyma are considered to be the most suitable features to deduce the position of enlarging zone at the time of marking. The variation of $\delta^{13}\text{C}$ values in preliminary studied species showed that isotopic approach can be applied and effective to deepen the understanding of wood formation in tropical rainforest trees. Therefore, the applications of DC-pulse marking and stable carbon isotope analysis are effective to study wood formation in tropical rainforest trees.

Monitoring of radial growth periodicity by dendrometer measurement, phenological observation and application of electrical impedance measurement in tropical trees were shown in Chapter 4. Each group of distinctiveness, classified by well-defined, poorly defined and absent growth rings meet the expected criteria. The poorly-defined group not always showed the seasonal rhythm of

radial growth and the absent group was sometimes showed seasonal radial growth. The well-defined group that grown in tropical monsoon and rainforest climates showed seasonal rhythm of radial growth and cambial activity when the trees slowing down their radial growth during the period of leaf shedding less precipitation. Therefore, the possibility of the occurrence of annual growth rings in well-defined group was expected.

Characterization of the growth ring and estimation of its annual formation in well-defined group was investigated by anatomical observation, stable carbon isotope, dendrometer measurement, phenological observation, and impedance measurement during in a known period. Annual formation of growth ring in well-defined species was estimated through the synchronicity among anatomical changes, variation in $\delta^{13}\text{C}$ values, seasonal rhythm of radial growth, cambial activity, leaf phenology, and precipitation. With all combined parameters, *P. canescens* in tropical monsoon area was clarified as forming an annual ring. *P. canescens* in tropical rainforest area is considered to have ambiguous or absent growth rings because it it grew under wet condition. *I. palembanica* was judged as having poorly-defined growth rings.

Therefore, in this study, the author concluded that *P. canescens* grown under tropical monsoon climate in Peninsular Malaysia can be promoted for use in tropical dendrochronology when the growth ring is considered to be formed annually. Meanwhile, the well-defined growth rings of *P. canescens*, *I. palembanica*, and other species with poorly-defined growth rings grown under tropical monsoon and rainforest climates should be clarified in the future with the addition of stable oxygen analysis, as for precise investigation of wood formation and confirmation of the annual ring formation in tropical rainforest trees.

Acknowledgment

The author would like to express his deepest gratitude to Assoc. Prof. Dr. Naoki Okada (Kyoto University) for his absolute supervision, inestimable comments and suggestions throughout this study. Special thanks to Prof. Dr. Akira Osawa (Kyoto University) comments made an enormous contribution to this study and critically examined this dissertation. The author extends special thanks to Prof. Dr. Keiji Takabe (Kyoto University) and Prof. Dr. Kanehiro Kitayama (Kyoto University) who critically examined this dissertation.

Special thanks and high appreciation to Emeritus Prof. Dr. Tadashi Nobuchi provides the author with invaluable guidance and comments, and warm encouragement. The author wishes to sincerely thank to Prof. Dr. Hamami Sahri (Universiti Putra Malaysia / Universiti Malaysia Sabah), Prof. Dr. Faridah Hanum Ibrahim (Universiti Putra Malaysia), lecturers in Faculty of Forestry, Universiti Putra Malaysia who gave him warm encouragements.

The author is grateful to Dr. Ahmad Zuhaidi Yahya (Forest Research Institute Malaysia), Dr. Mohd. Zaky Abdullah (Forest Research Institute Malaysia), Prof. Dr. Faridah Hanum Ibrahim (Universiti Putra Malaysia), and Dr. Paiman Bawon (Universiti Putra Malaysia) for their permission to conduct field research and providing great supports.

The field experiments were greatly assisted by Mr. Mohd. Ramly Mohd. Salleh Forest Research Institute Malaysia), Mr. Kamis Mat Ain (Forest Research Institute Malaysia), Mr. Sukeri Don (Forest Research Institute Malaysia), Mr. Mohd Fakhruddin Imam Supaat (Universiti Putra Malaysia), Mr. Ahmad Ridhwan Isa (Universiti Putra Malaysia), Mr. Mohd Kamil Ismail (Universiti Putra Malaysia), and other staffs.

The author is also grateful to Assist. Prof. Dr. Masako Dannoura, and all the members who shared precious time in Forest Utilization Seminar.

The author would like to thank the Sasakawa Scientific Research Grant from Japan Science Society for a grant that made it possible to complete this study.

Finally, the author wishes to express his most sincere appreciation to his family and friends for their love, encouragement, motivation, and total devotion throughout the entire study period.

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Curriculum Vitae

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