

## SEASONAL VARIATIONS IN CAMBIAL ANATOMY OF PLANTATION-GROWN *AZADIRACHTA EXCELSA*

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**WANG KH, NOBUCHI T, ABDUL AZIM AA & SAHRI MH. 2013. Seasonal variations in cambial anatomy of plantation-grown *Azadirachta excelsa*.** Tropical trees do not have clear growth rings as they do not show clear radial growth periodicity. It is important to investigate the cambial structure and anatomical characteristics of cells derived from cambium in order to understand the characteristics of radial growth periodicity. A comparison of the anatomical characteristics of cambium in *Azadirachta excelsa* was carried out between actively and non-actively growing seasons. Wood blocks comprising bark, cambium and wood were collected from a living tree. Sections were cut from epoxy-embedded block using a microtome. A periodic acid-Schiff's method was used for staining. The results revealed that the average numbers of cambial and enlarging cambial zone cells varied at different months. During the wet season, the cambium formed high number of wood cells. During the dry season, from November till February, the cambium was inactive. The cambial zone was active for most period of the year. This finding indicates that the cambial growth of *A. excelsa* is sensitive to environmental factors, particularly rainfall.

Keywords: Tropical trees, cambial activity, growth ring, radial growth

**WANG KH, NOBUCHI T, ABDUL AZIM AA & SAHRI MH. 2013. Variasi bermusim dalam anatomi kambium pokok *Azadirachta excelsa* yang ditanam di ladang.** Pokok tropika tidak mempunyai gelang pertumbuhan yang jelas kerana pokok tidak menunjukkan pertumbuhan jejari bermusim yang jelas. Kajian tentang struktur kambium dan ciri anatomi sel yang berasal daripada kambium adalah penting untuk memahami ciri pertumbuhan jejari bermusim. Perbandingan ciri-ciri anatomi kambium pokok *Azadirachta excelsa* antara musim pertumbuhan aktif dan musim pertumbuhan tidak aktif dijalankan. Penyelidikan dijalankan dengan mengambil bahagian yang mengandungi kulit, kambium dan kayu daripada sampel pokok hidup. Kayu yang dirawat dengan resin epoksi dihiris menggunakan mikrotom. Kaedah Acid-Schiff digunakan untuk pewarnaan. Keputusan menunjukkan bahawa purata jumlah sel kambium dan sel zon kambium yang membesar adalah berbeza pada bulan yang berlainan. Pada musim hujan, kambium menghasilkan jumlah sel kayu yang banyak. Pada musim kering, dari bulan November hingga bulan Februari, kambium tidak aktif. Zon kambium menunjukkan pola aktif pada kebanyakan masa sepanjang tahun. Keputusan menunjukkan bahawa pertumbuhan kambium pokok *A. excelsa* adalah sensitif kepada faktor alam sekitar terutamanya hujan.

### INTRODUCTION

Wood formation in trees is a periodic process. The process is regulated by both internal signals and environmental factors (Marion et al. 2006, Begum et al. 2008). The periodicity of radial growth in temperate trees is very clear compared with tropical trees (Nobuchi & Sahri 2008). This is because trees in temperate climates have clear annual growth rings. However, some tropical species have been reported to have more or less distinct growth ring boundaries (Worbes 2002, Dezzeo et al. 2003, Brienens & Zuidema

2005). Although some tropical trees exhibit clear growth rings, their ring boundaries are not always distinct and their seasonal characteristics are often not known (Nobuchi et al. 1995).

Seasonal characteristics of vascular cambium in temperate species have been extensively studied (Rao & Rajput 2001, Rensing & Samuel 2004). The growth patterns of tropical trees which include their initiation and cessation are still not well understood (León-Gómez & Monroy-Ata 2005). In order to have a thorough

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understanding of the characteristics of the radial growth periodicity of tropical trees, it is important to investigate the cambial structure and anatomical characteristics of cells derived from cambium.

The objectives of this research were to investigate anatomical characteristics of cambial and enlarging zone cells and to compare the anatomical features between actively and non-actively growing season. Plantation-grown *Azadirachta excelsa* was selected as the experimental species. It is easily available. Information on the radial growth of *A. excelsa* would be essential in managing forest plantations to overcome timber shortage as this plantation species is valued for home construction and furniture (Trockenbrodt et al. 1999). The ring structure of *A. excelsa* is characterised by a band of axial parenchyma, surrounded by fibre cells with wall thinner towards the bark compared with the pith. The vessels of the wood are mostly solitary and diffuse-porous in various sizes.

## MATERIALS AND METHODS

### Study site and climate data

The Mata Ayer Research Station of the Forest Research Institute Malaysia, located in the north of Peninsular Malaysia, was selected as the study site. The site is a plantation forest which experiences clear dry and wet seasons. It is considered a tropical seasonal forest. Data on the monthly total rainfall and mean temperature were collected from the Chuping meteorological station (6°29' N, 100°16' E). Figure 1 shows one-year climatic factors of north Peninsular Malaysia.

### Sample collection and preparation

A 12-year-old *A. excelsa* tree was used. The height of the tree was 12.3 m and the diameter at breast height was 21.3 cm. Wood blocks comprising the inner bark, cambial tissues and outer sapwood were collected from the main trunk at breast height. Collections of samples were made in August and November 2008 as well as February and May 2009. The sampling

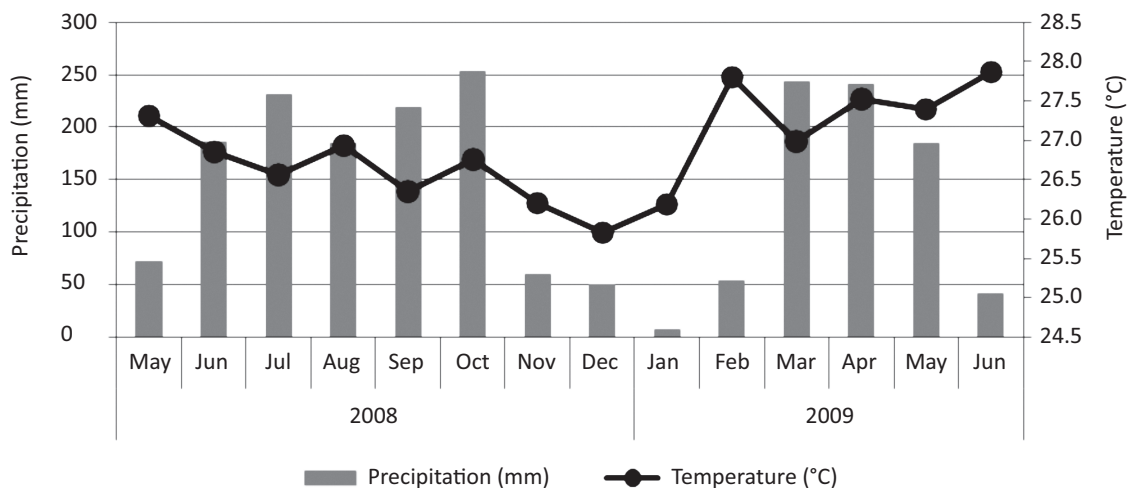
dates were at the middle of the wet season, beginning of the dry season, end of the dry season and after the dry season. Only four blocks were collected to ensure as little injury to the sampled tree. The size of the collected wood blocks was 2 cm × 2 cm × 2 cm. The wood blocks were fixed in 3% glutaraldehyde for preservation. The samples were reduced to 2 mm × 2 mm × 10 mm before embedding in epoxy resin. Transverse sections with thickness of 20–25 µm were cut from the epoxy-embedded blocks using a sliding microtome (Nobuchi & Sahri 2008). They were stained using the periodic acid-Schiff's (McManus 1948) technique to distinguish the different stages of cell development. The changes from thin-walled cambial cells to thick-walled secondary xylem were observed under the microscope. Sections were mounted in Canada balsam to prepare permanent slides.

### Laboratory analysis

A light microscope under bright field and polarised light with the aid of an image processing and analysis system was used to observe the anatomical features of the cambium and xylem. To evaluate the anatomical characteristics of the cambial zone and cells derived from it, the cambial zone and enlarging cells were measured along 30 cell rows observed on the transverse sections. The counting method of the number of cambial and enlarging zone cells was according to Nobuchi and Hori (2008). A greater number of the cambial and enlarging zone cells were assumed to indicate greater cambial activity (León-Gómez & Monroy-Ata 2005). The counting is described below.

#### *Cambial zone cell*

Cambium, consisting of cambial initials, is a single cell layer surrounding a tree between its bark and wood. In a radial cell file from bark to wood, only one cell is theoretically considered as cambial initial. The term cambial zone describes the group of cells which includes cambial initials and cells with ability to divide (Schmid 1976). In this research, cambial



**Figure 1** Monthly rainfall and monthly average temperature of north Peninsular Malaysia

zone cells refer to the radially flattened cells between the inner phloem and outer xylem (Nobuchi & Hori 2008).

#### *Enlarging zone cell*

Enlarging zone cells (short enlarging cells) are cells that start to increase in diameter and show intrusive growth beyond the cambial zone cells in the transverse section. After the cessation of enlarging zone cells, secondary wall formation begins and the boundary between them can be clearly seen under a polarised light microscope. This is because cells with secondary wall formation show bright light in the transverse section. Enlarging cells refer to cells that are next to the cambial zone (Nobuchi & Hori 2008).

Figure 2 shows the cambial and enlarging zone cells in the transverse section while Figure 3 illustrates the transverse section of the cambial zone and enlarging cells under bright field and polarised light microscopes.

#### **Terminology**

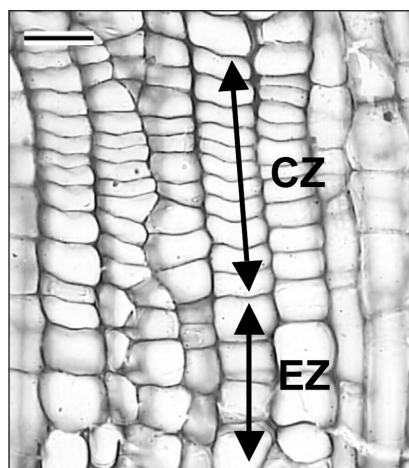
Active cambium means high number of cells in the cambial zone and the presence of the newly-formed xylem (and phloem) cells. Inactive cambial activity means minimum number of cells in a cambial zone and no newly-formed xylem and phloem cells.

#### **RESULTS**

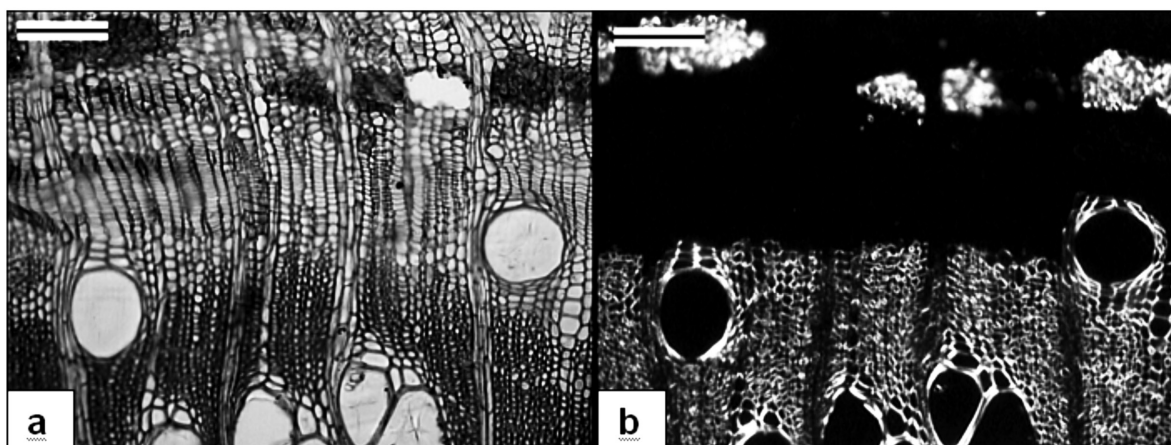
Table 1 summarises the numbers of cambial and enlarging zone cells observed in August, November, February and May. The formation of vessel element results in earlier enlargement of vessel in cell rows compared with those without vessel (Wakuta et al. 1973). Therefore, it is important to consider the effect of vessel formation when counting the number of cells. Table 1 shows data of the cell rows, with and without vessel.

#### **Seasonal variation of the cambial activity**

Figure 4 shows the light micrographs of cambial and enlarging zones of *A. excelsa* in August, November, February and May. At different months, *A. excelsa* presented different average numbers of cells. In August 2008, *A. excelsa* showed the greatest number of cambial and enlarging zone cells (Table 1). During this month, wide cambial zone was formed. Active cambial activity was identified by the presence of new cell plates, indicating that the cambium was active (Figure 4a). In November 2008, the cambium zone decreased in cell number. In other words, fewer cell layers were formed in the cambial and enlarging zone cells compared with August. Although cambium showed decreased activity, anticlinal divisions were formed (Figure 4b). In February 2009,



**Figure 2** Transverse section showing cambial and enlarging zone cells; CZ = cambial zone cell; EZ = enlarging zone cell; scale bar = 30 µm



**Figure 3** Transverse sections showing location of cambial and enlarging zone cells: (a) conventional light micrograph, (b) polarised light micrograph; scale bar = 200 µm

**Table 1** Numbers of average cambial and enlarging zone cells of *Azadirachta excelsa*

Month	Vessel	Average number of cambial zone cell	Average number of enlarging zone cell	Total
August	Without	14	29	43
	With	9	23	32
November	Without	10	20	30
	With	7	15	22
February	Without	5	10	15
	With	5	8	13
May	Without	8	18	26
	With	6	19	25



the average numbers of cambial and enlarging zone cells showed the lowest cell number. Cambial activity was considered inactive but not dormant. This was because the cell number in the enlarging phase did not decline to zero (Figure 4c). Cambium was reactivated in May 2009 with the formation of new cell plates (Figure 4d). The cambial and enlarging zones increased in cell numbers.

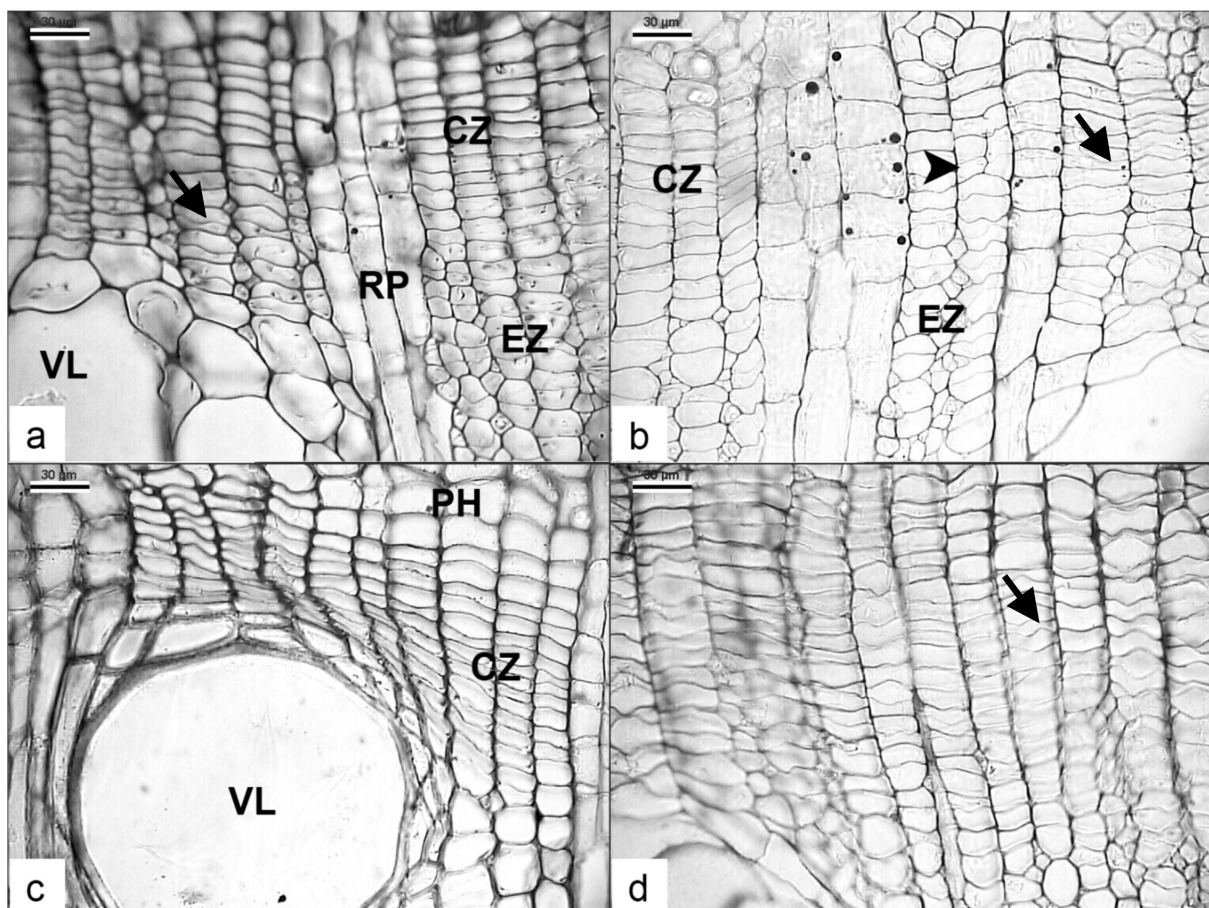
Figure 5 shows the relationship between seasonal changes of cambial and enlarging zone cells of *A. excelsa* and precipitation. In particular, the sampled tree exhibited active and inactive phases in a year.

## DISCUSSION

Periodicity of cambium can be controlled by internal signals and environmental factors (Begum et al. 2008). However, this research

only focused on the active and inactive periods of cambial activity in relation to the variation in climatic factors such as temperature and rainfall. The results showed that the tree did not grow at the same rate. The cambial and enlarging zones of the sampled tree showed seasonality with tendency towards increase in cell number during the wet season and tendency to decrease during the dry season. Rainfall was reported to play a major role in tree growth in the tropical forest (Volland-Voigt et al. 2011). Although the results of the current study did not provide enough data on the seasonal tree growth behaviour of *A. excelsa*, it documented seasonal cambial structure.

Active cambial activity was shown in August, i.e. during the middle of the wet season. Cell divisions were active, evidenced by the formation of new cell plates, thus widening the cambial zone. This phenomenon is



**Figure 4** Light micrographs showing cambial zone and enlarging zone in *Azadirachta excelsa*: (a) August, (b) November, (c) February and (d) May; PH = phloem, CZ = cambial zone, EZ = enlarging zone, VL = vessel, RP = ray parenchyma; arrow head indicates anticlinal division; arrow indicates cell plate formation; scale bar = 30 µm

considered to be influenced by high water availability during high rainfall period. Marcati et al. (2008) stated that there were many immature xylem cells differentiating when cambial cells were active and that cambial activities were correlated with the wet season. The cambium showed decrease in activity when the weather condition became relatively dry during the onset of the dry season in November. A similar observation was found in *Pinus merkusii* and *P. kesiya* that grew in Thailand. The cambial activity of the trees was found to have gradually declined towards the onset of the dry season and decreased from November till February, which was the dry season (Pumijumnong & Wanyaphet 2006).

In February, which was the end of the dry season, the sampled tree showed inactive cambial activity. The cell walls of the cambial zone were relatively thick compared with the other months. The cambial zone was narrow and surrounded by secondary xylem and phloem. This phenomenon was also noticed in *Schizolobium parahyba* during cambial inactive condition (Marcati et al. 2008). The inactive condition of the cambial zone seemed to be associated with water stress because there was reduction in rainfall during the dry season although temperature was high enough for its activity. There have been reports that tropical areas which experience reduced precipitation of several months face cambial dormancy (Worbes 2002, Schongart et al. 2006).

The cambium was reactivated in May during the wet season. The division and differentiation of cambial zone cells occurred with increase in rainfall. Similarly, the cambial growth of *Azadirachta indica* showed the same tendency (Rao & Rajput 2001). During this month, the cambial cells swelled and consisted of thin cambial cell walls. The bark of the sampled tree separated relatively far from the wood as compared with the condition in February. These indicated the beginning of cambium activity. Thus, rainfall is considered one of the factors to reactivate and enhance cambial activities after the dry season.

Studies carried out on various species in different countries have reported temperature as an important climatic factor influencing cambial activity. However, the current study has shown that variation in temperature

has less effect. Constant temperature is an unlikely factor determining cambial activity (León-Gómez & Monroy-Ata 2005). The area in the present study showed almost constant temperature around 26.9 °C. Hence, this study suggested that active and inactive cambial activities were influenced by rainfall. Most studies have shown a correlation between tropical tree growth and rainfall as tropical areas generally show little interannual variation in temperature (Rozendaal & Zuidema 2011). However, for some tropical species, relationships exist between growth and temperature as well as growth and precipitation (Buckley et al. 2007, López & Villalba 2010).

Unlike other tropical trees, *A. excelsa* has growth rings as a result of seasonal cambial activities during the wet and dry seasons. The width of the rings varies with environmental conditions.

## CONCLUSIONS

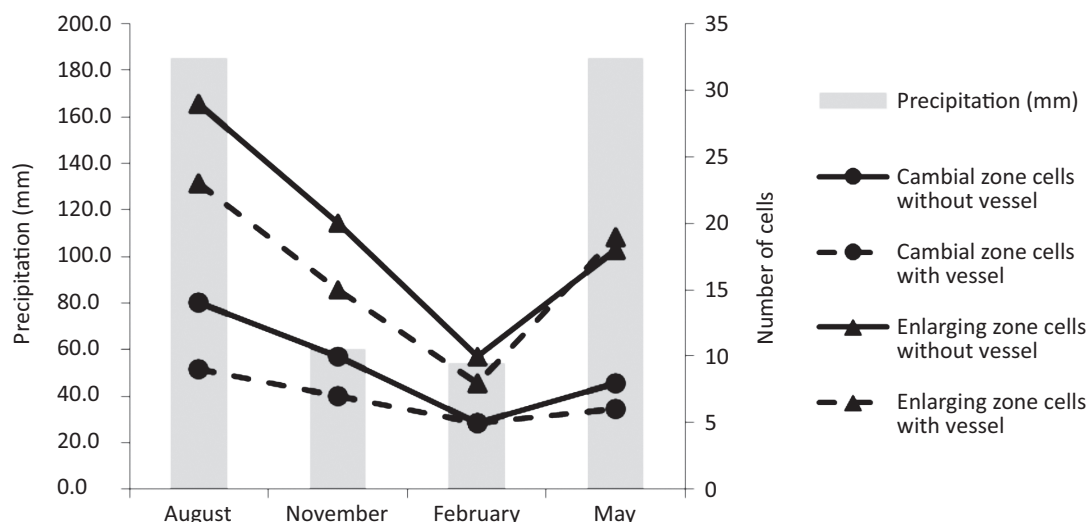
The average numbers of cambial and enlarging zone cells showed significant increases during the rainy season, indicating greater cambial activity. *Azadirachta excelsa* is believed to have formed growth rings as a result of periodical growth during the rainy and dry seasons.

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**Figure 5** The relationship between monthly precipitation and average number of cells for rows with and without vessels

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