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Original article

Relationship between seasonal cambial activity, development of xylem and phenology in *Azadirachta indica* growing in different forests of Gujarat State

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Abstract – Seasonal cycle of cambial activity was compared among the trees of *Azadirachta indica* growing in Moist Deciduous (MDF), Dry Deciduous (DDF) and Scrub land Forest (SF) of Gujarat State. Radial growth occurred in two growth flushes in MDF and DDF. Cambial cell divisions in MDF started in February and June resulting maximal radial growth in August-September when the rains were heavy and ceased in January and May during the drier part of the year. In DDF the first flush of growth commenced in January with maximal xylem development in April and ceased in May. The second flush of cambial activity began in June with the arrival of rains, reached peak in October and ceased in December. Cambium was active throughout the year in SF and attained its peak activity thrice i.e. in February, July and October. With complete maturation of leaves in November, the cell divisions were rather slow in MDF and SF whereas no divisions were encountered in DDF. Cambial rays exhibited large intercellular spaces during drier months in all the three forests. Seasonal behavior of vascular cambium was discussed in relation to phenology and local climatic conditions.

***Azadirachta* / intercellular spaces / vascular cambium / xylem**

Résumé – Relation entre l'activité saisonnière du cambium, la croissance radiale du xylème et la phénologie chez *Azadirachta indica* dans différents types d'écosystèmes forestiers de l'état du Gujarat. Les cycles saisonniers de l'activité cambiale chez *Azadirachta indica* dans différents types d'écosystèmes forestiers de l'état de Gujarat ont été comparés entre des arbres provenant de forêts humide décidue (MDF), sèche décidue (DDF) et de savane arborée (SF). La division des cellules cambiales commence en février et juin pour les MDF, ce qui se traduit par une croissance radiale maximale en août-septembre, au moment des fortes pluies, et un arrêt en janvier et mai pendant les périodes les plus sèches de l'année. Dans les DDF, le premier démarrage de croissance radiale commence en janvier, avec un maximum en avril et un arrêt en mai. Une seconde période d'activité commence en juin avec l'arrivée des pluies, atteint son maximum en octobre et s'arrête en décembre. Dans les SF le cambium reste actif toute l'année avec trois pics d'activité en février, juillet et octobre. Lorsque les feuilles arrivent à maturité complète en novembre, la vitesse de division cellulaire du cambium est relativement faible dans les MDF et le SF et nulle dans les DDF. Dans le cambium, les rayons présentent des espaces intercellulaires importants pendant les mois secs pour les trois types de forêts. Le fonctionnement saisonnier du cambium est ensuite discuté en relation avec la phénologie et les conditions climatiques locales.

***Aazadirachta* / activité cambiale / climat / phénologie / xylème**

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1. INTRODUCTION

Seasonal behavior of vascular cambium in temperate species has been studied widely than tropical ones but relevant studies have been made by earlier workers [1, 8, 9, 22, 26, 29]. A few experimental studies have also been made to understand the effect of various climatic factors (viz. temperature, rainfall, photoperiod etc.) under controlled environment [17, 18, 31]. However, the response of vascular cambium in tropical trees species growing naturally under different climatic conditions is not yet fully understood.

*Azadirachta***,** a moderately fast growing tree has multiple domestic uses, medicinal value and many other commercial exploitable byproducts [3]. Besides its economic importance, no information is available on its seasonal behavior of vascular cambium. Our previous studies showed significant variations in the seasonal activity of the same species growing naturally under different climatic conditions [25, 29]. Therefore, present study was aimed to understand the behavior of cambium in *Azadirachta* growing naturally under local climatic conditions in different forest types ranging from moist deciduous to pure desert conditions.

2. MATERIALS AND METHODS

Samples of cambial tissues together with inner bark and outer sapwood were collected from the main trunk at breast height of 15–20 years old trees of *Azadirachta indica* having similar trunk diameter. These trees were naturally growing in moist deciduous forest (MDF) at Waghai in Dangs, dry deciduous forest (DDF) at Pavagadh, and Scrubland forest at Bhuj and Nakhatrana in Kutch. Periodic collections were made during the second week of every month from January to December 1994. Two trees were sampled each time to obtain four blocks and no tree being sampled more than once. The blocks measuring about 60 × 20 mm were excised with the help of hammer, chisel and grafting knife and fixed immediately in FAA [4]. Suitably trimmed small pieces of these blocks were sectioned in transverse, radial and tangential longitudinal planes at 15 to 20 μm thick on a sliding microtome. After staining with tannic acid-ferric chloride-lacmoid combination [6], sections were mounted in DPX after passing through ethanol-xylene series.

The terms cambial zone and cambium are used to include the entire population of ray and fusiform cambial cells between the xylem and phloem. Cambial activity was determined by counting the number of undifferentiated layers of cambium lying between xylem and phloem in transverse sections. The terms cambial inactivity or rest are used to define the suspension of cell division activity anywhere within the cambial zone. One hundred measurements of cambial layers were selected randomly to obtain the mean and standard deviation.

Seasonal phenological changes of the trees were recorded at the time of each sample collection. Data on air temperature and rainfall were obtained from the Indian Meteorological Department, Ahmedabad.

3. RESULTS

3.1. Structure of cambium

The cambium is nonstoried with vertically elongated, randomly distributed fusiform cambial cells and horizontally arranged isodiametric ray cambial cells. When dormant fusiform cambial cells have thick radial walls with beaded pattern and thinner with less beaded pattern when active. Ray cambial cells are turgid polygonal and compactly arranged in active cambium (*figure 1A*) but they become flaccid showing prominent intercellular spaces (*figure 1B*) when the cambium becomes inactive. The intercellular spaces are relatively more prominent in MDF and DDF. The length of fusiform cambial cells ranges from 291 to 440 μm and 17 to 23 μm in width. Cambial rays are uni-multiseriate and 165 to 361 μm and 47 to 87 μm in height and width respectively.

3.2. Cambial activity

In MDF cambium remains active for major part of the year except in January and May. It reaches peak in August-September with 12–16 layers of cells in each radial file, while in May narrow cambial zone is found surrounded by mature xylem and phloem elements (*figure 2A*). Radial growth in the trees of DDF occurs in two distinct growth flushes. The first flush of cambial activity starts in January and reaches peak in April (*figures 2B, C*). In May cell divisions cease and cambial zone remains surrounded by mature xylem and phloem. The second flush of activity starts in June resulting wide cambial zone surrounded by differentiating vascular elements

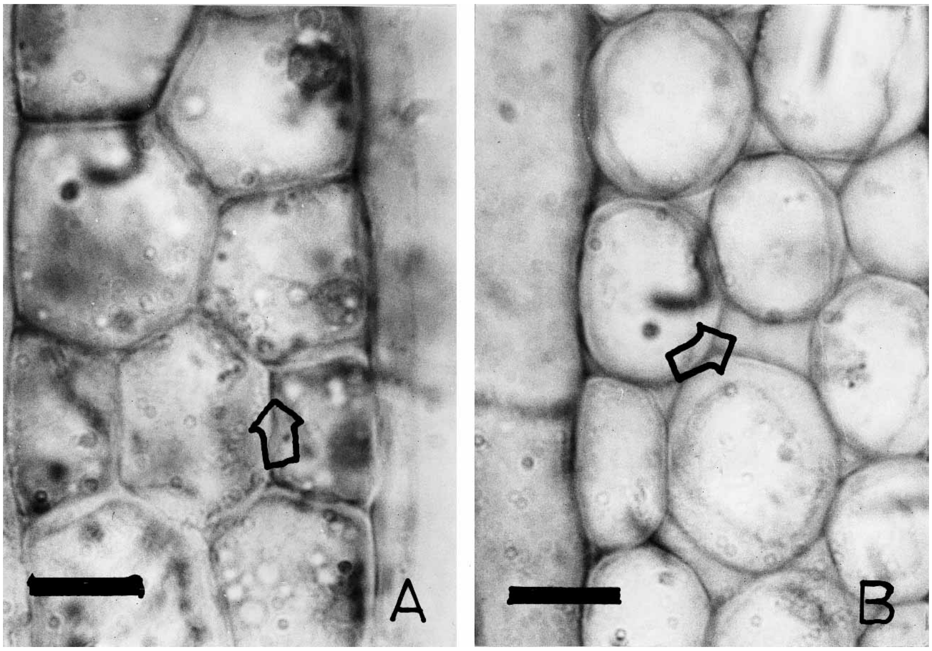

Figure 1. Tangential longitudinal view of cambium in *Azadirachta indica*. A: Cambial ray showing compactly arranged cells during growing season (arrow). B: Cambial ray showing large intercellular spaces during rest condition (arrow). Scale bar = 25 μm.

from July to October. Then the cambial cell division and differentiation declines in November and ceases in December (*figure 2D*). In SF, cambial cell divisions continue throughout the year. However, the activity reaches peak with 10-17 number of cells in the cambial zone in February, July and October (*figure 2E*).

3.3. Cambial activity in relation to phenology

Azadirachta being the semi-evergreen tree does not shed all the leaves at a time. In all the three forests, defoliation starts first on the upper most branches and spreads gradually towards lower ones followed by sprouting of new leaves on the top branches. Defoliation begins in November and the entire crown of old leaves is replaced by new crop of leaves by March in both MDF and SF while in DDF it occurs in February followed by fruit setting and maturation in the succeeding months. However, fruit ripening and dispersal occurs in May-June in all the three forests. A second flush of flowering in September-October followed by fruit setting is noticed in SF.

In DDF, periclinal divisions in the cambial zone initiate in January with the sprouting of new leaves in December. Similarly the first peak of radial growth in SF coincides with the development of new leaves in February. Although the entire crown of old leaves is replaced by young ones in February-March, cambial cells cease to divide in May in MDF and DDF. Whereas, in DDF the cessation of cell divisions and differentiation of xylem and phloem in December coincides with leaf yellowing in October-November. In MDF and SF cell divisions in the cambial zone are found to be sluggish during the initiation of defoliation (*table I*).

3.4. Cambial activity in relation to climatic factors

Cambial cells begin to divide in June in MDF at the end of drier part of the year, whereas, in DDF divisions commence in January. In all the three forests, cambial activity and differentiation of xylem reach peak in monsoon (July-September). Activity declines with the last shower of rains in October-November and cambial cells cease to divide in May when water is scarce in both MDF and DDF (*figures 3A, B*).

In DDF, first flush of cambial activity initiates in January when the air temperature is reported minimum for the year. Cambial cell divisions and development of xylem are found suspended in May in DDF and MDF only

Table I. Periodic changes in the number of cambial layers in relation to phenology in *Azadirachta indica* A. Juss growing in MDF, DDF and SF of Gujarat state.

Month	MDF	Cambial layers	DDF	Cambial layers	SF	Cambial layers
JAN	Leaf yellowing, sprouting of new leaves, initiation of defoliation	5 ± 1.93	New leaves, development of floral bud	8 ± 1.95	Yellowing of leaves, initiation of defoliation	8 ± 0.72
FEB	Sprouting of new leaves, defoliation in progress	9 ± 1.30	New leaves, flowering	7 ± 1.69	Defoliation, sprouting of new leaves, flowering	10 ± 0.74
MAR	New leaves, flowering	10 ± 1.30	New leaves, flowering	8 ± 1.91	New leaves, flowering, fruit setting	9 ± 1.50
APR	New leaves, flowering, Fruit setting	8 ± 1.11	New leaves, fruit setting	10 ± 2.27	New leaves, flowering, fruit setting	9 ± 1.35
MAY	New leaves, fruit maturation	5 ± 0.78	Full foliage, fruit maturation, dispersal	6 ± 1.32	New leaves, fruit maturation	11 ± 1.98
JUN	Sprouting of new leaves, fruit dispersal	10 ± 1.56	Full foliage, fruit dispersal	8 ± 1.67	New leaves, fruit dispersal, flowering	12 ± 1.32
JUL	New leaves, full foliage	10 ± 1.32	Full foliage, terminal bud active	7 ± 0.75	New leaves, terminal bud active, flowering	17 ± 2.30
AUG	New leaves, full foliage	12 ± 1.69	Full foliage, flowering in some branches, terminal bud active	9 ± 1.45	Terminal bud active, fruit setting	10 ± 1.63
SEP	Full foliage, terminal bud dormant	16 ± 2.51	Leaf maturation, terminal bud dormant	9 ± 1.63	Terminal bud dormant, fruit maturation	12 ± 1.74
OCT	Leaf maturation	9 ± 1.16	Leaf maturation and yellowing	14 ± 2.68	Fruit dispersal, mature leaves	14 ± 1.32
NOV	Leaf maturation	9 ± 1.16	Leaf yellowing and initiation of defoliation	8 ± 1.32	Mature leaves	10 ± 2.00
DEC	Leaf yellowing	7 ± 1.10	Defoliation, sprouting of new leaves	5 ± 0.72	Leaf yellowing	11 ± 2.17

when the temperature remains maximal for the year. Although cambial cells start dividing in June in MDF, rapid divisions and differentiation of xylem occurs only after the shower of rains in June.

However, no such correlation is observed between cambial activity and climatic factors in trees growing in SF.

3.5. Development of vascular elements

Xylem development precedes that of phloem and phloem development ceases first followed by xylem in both MDF and DDF while in SF differentiation of both the tissues continue throughout the year.

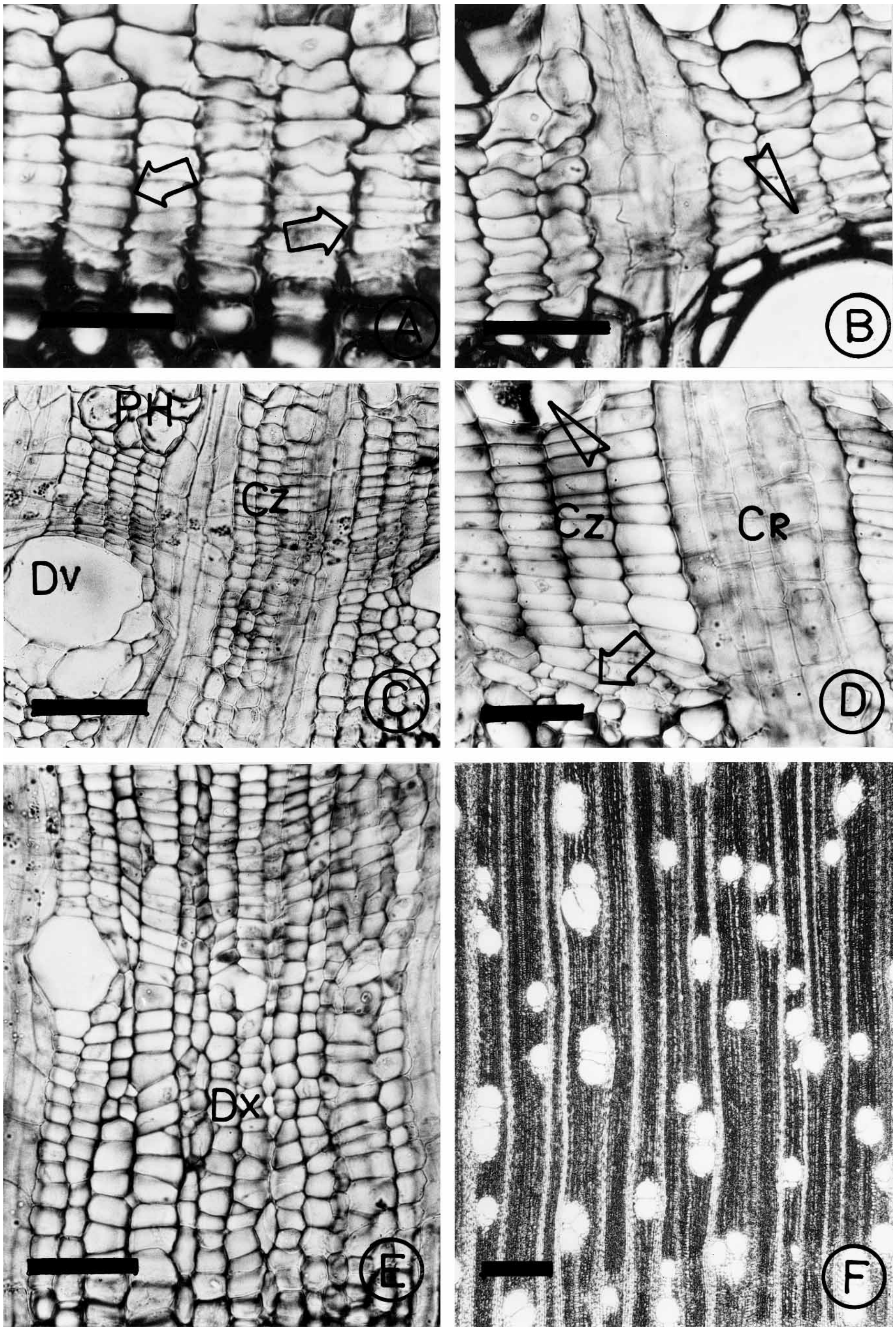

Figure 2. Transverse view of cambium with adjacent xylem and phloem in *Azadirachta indica*. A: Inactive cambium in May flanked by mature xylem and phloem showing thick radial walls (arrows) in MDF. B: Initiation of cambial cell division in January in DDF. Arrowhead indicates newly formed thin tangential walls. C: Wide cambial zone in April in DDF. Note the differentiating xylem and phloem elements. D: Cessation of cell division in the cambial zone during November while the maturation of phloem (arrowhead) xylem (arrow) continue in DDF. E: Peak activity of cambium in July with many differentiating xylem elements in SF. F: Structure of xylem in MDF. CZ: Cambial Zone, CR: Cambial Ray, DV: Differentiating Vessel; DX: Differentiating Xylem; PH: Phloem. Figures 2A–E: Scale bar = 75 µm. Figure 2F: Scale bar = 100 µm.

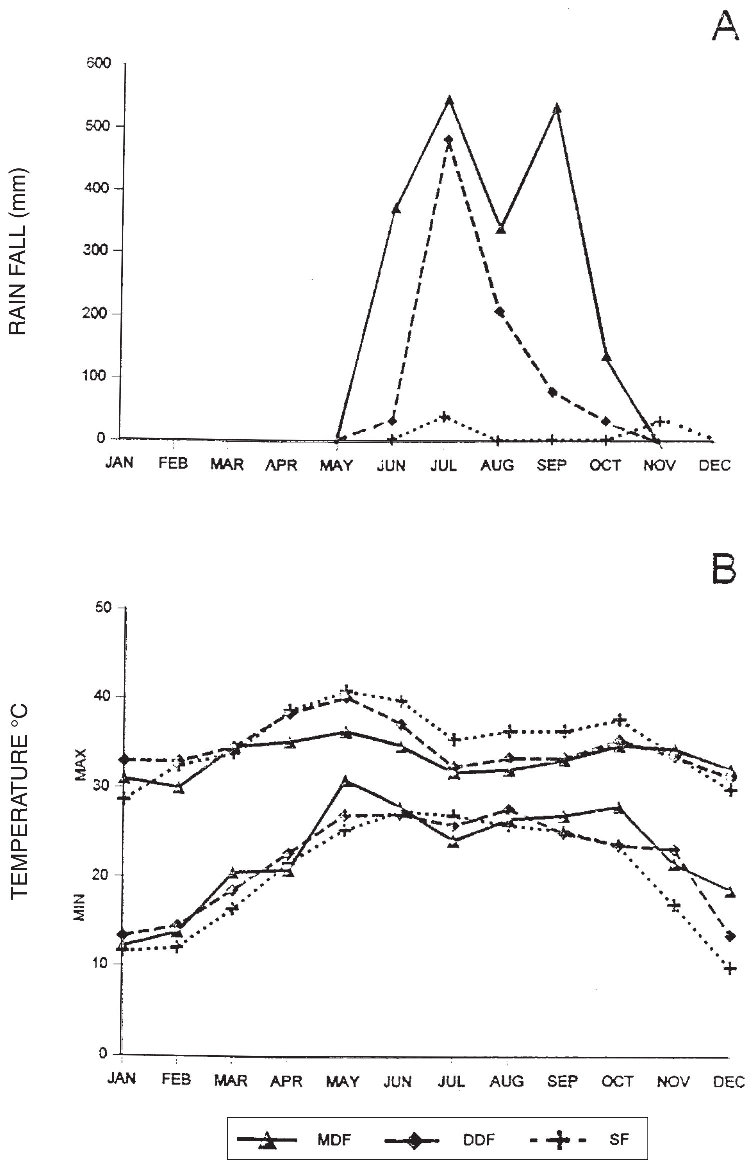

Figure 3. Graphic representation of average rainfall (A) and temperature (B) in MDF, DDF and SF recorded at Indian Meteorological Center, Ahmedabad in the year 1994.

Cambial growth starts in June in MDF and culminates in August-September with 20–30 and 3–5 differentiating xylem and phloem elements respectively. Cell division declines gradually and ceases in January and May. In DDF radial growth occurs in two growth flushes, the first flush of activity reaches peak in April and the second flush in October with 16–20 and 2–4 differentiating xylem and phloem elements respectively. Radial growth continues throughout the year in SF with peak growth occurring in February, July and October. However, radial growth is relatively more in July compared to that of February and October.

Xylem is diffuse porous with indistinct growth rings in all the three forests (*figure 2F*). No much variation is observed in the xylem structure of trees growing in MDF and DDF. However, in SF, vessel diameter is less but their frequency remains relatively more compared to that of other two forests. The length of vessel elements vary from 229–311 μm in MDF, 249–307 μm in DDF and 232–285 μm in SF. Similarly vessel diameter ranges from 113–170 μm in MDF and DDF whereas, 98–153 μm in SF. Vessel frequency per 0.5 mm^2 area of xylem in transverse view vary from 13–16 in MDF, 8–14 in DDF and 15–22 in SF.

4. DISCUSSION

In temperate conditions onset of cold period coincides with the cambial inactivity [5, 23]. Similarly in mediterranean climate dry and warmer summer inhibits the growth leading to cambial rest [16, 18, 20]. However, in tropical trees the period of activity is relatively longer, where the radial growth continues either throughout the year [9, 10] or for the major part of the year [7, 9, 11, 29]. In *Azadirachta* cambial growth occurs for the major part of the year in MDF and DDF whereas in SF it occurs throughout the year.

In MDF, cambial activity declines with leaf maturation and yellowing and ceases in January and May, although young leaves sprout on the branch tips. In DDF cambial cell division and differentiation of xylem cease in November-December following maturation and yellowing of leaves October-November. while in SF, inspite of the adverse conditions the phenological pattern of the trees is more or less similar to those growing in the MDF and DDF and the cambium maintains its activity throughout the year. Cambial cell division and differentiation of xylem is found suspended in May in MDF and DDF. As a semideciduous tree, there is no direct correlation between cambial activity and phenology in *Azadirachta*. It seems true that the effect of buds and new leaves on cambial activity is considered to be more local in evergreen trees than deciduous ones [12].

The intensity of cambial activity is controlled by various physiological and environmental factors. Temperature is known to play important role in activating the cambium [13, 29]. It is well accepted that day length and high temperature influences the shoot growth through its effect on seasonal distribution of bud expansion into shoots while onset of cold conditions and short days usually brings about the dormancy in temperate species [15]. But in many tropical species cambium remains active for the major part [9, 11, 27] or throughout the year [8, 10].

In DDF, divisions in cambial cells initiate in January when the temperature is lowest of the year, on the other hand, cambium tends to be inactive in May in MDF and DDF, when the temperature recorded is maximum for the year. Interestingly, even at the highest air temperature cambial cell divisions and xylem development continues in SF.

Rainfall has direct bearing on the enhancement of cambial activity [14, 27, 28, 29]. Cambial growth of woody plants is exceedingly sensitive to and inhibited by water deficits [2, 15]. The water stress inhibits the divisions by reducing the turgor pressure of the cambial cells and indirectly inhibits the cambial activity by reducing the growth of the leaves and apical meristem thereby affecting the supply of hormones and assimilates required for the process [19, 30]. The temporary inhibition of cambial growth in May in MDF and DDF may be associated with the water stress. Rapid divisions and differentiation of its derivatives occurs with the arrival of rains in June and differentiation of xylem and phloem culminates in monsoon when the rains are heavy. Similar observations are also made by Fahn et al. (1968). Furthermore, the occurrence of large intercellular spaces in the cambial rays during drier part of the year and their disappearance with the onset of rains in June also confirms the water stress during the cambial rest. [24].

It is interesting to note that cambium remains active even during the summer months in SF, which experiences relatively little precipitation and higher temperature. Similar behavior of cambium has been noticed in *Acacia* species growing in desert conditions [2, 12]. The possible explanation is that those trees that have access to water all the year around would show continuous cambial activity. This could be possible because of deep root system capable of tapping underground water. It appears that *Azadirechta* species growing in SF may adapt to desert conditions where rainfall is scanty and fluctuations in temperature are more throughout the year. On the other hand, in MDF and DDF rainfall is relatively higher and no much variation exists in day and night temperatures. Although cambial growth occurs for major part of the year in all the three forests, peak activity occurs only in August-September in MDF, April and October in DDF and February, July and October in SF. This indicates that *Azadirachta* is more adapted to drier conditions than the moist ones.

According to Liphschitz et al. [16, 17] the periodicity of cambium is endogenously determined, external factors like temperature and water supply may advance and/or prolong the growing phase but do not prevent alternations between growing and resting phase. It is true that correlation between external and internal factors, and activity of cambium does not necessarily mean a simple relation between the two [21]. Present study on *Azadirachta* growing in different forest types indicate that trees develop different adaptive strategies in response to the local climatic conditions.

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