| Phyton (Horn, Austria) | **Vol. 51** | Fasc. 2 | 289–297 | 20. 12. 2011 |
|---|---|---|---|---|

# Development of Vascular Cambium in the Petiole of *Terminalia catappa* L. (*Combretaceae*)

By

Kishore S. RAJPUT*) and Karumanchi S. RAO*)

With 2 Figures

Received November 26, 2008

Accepted June 30, 2011

Key words: Cambium, procambium, metacambium, *Terminalia catappa*.

Summary

RAJPUT K. S. & RAO K. S. 2011. Development of vascular cambium in the petiole of *Terminalia catappa* L. (*Combretaceae*). – Phyton (Horn, Austria) 51 (2): 289–297, with 2 figures.

Structure and development of vascular cambium and pattern of secondary growth were studied histologically in the petiole of *Terminalia catappa* L. (*Combretaceae*). The petiole cambium is non-storied with vertically elongated fusiform and small isodiametric ray cambial cells. After the formation of few protoxylem and protophloem elements from procambium, periclinal divisions in metacambium produced metaxylem and metaphloem. Compared with main shoot, the differentiation of interfascicular cambium and continuity between fascicular and interfascicular cambium was rather slow. The procambial strands became more prominent in the second leaf giving rise to elements of metaxylem and metaphloem. In the third leaf tangential continuity between metacambial cells became more prominent with inception of interfascicular cambium between the bundles. The cambium remained active until the leaf reached senescent stage. With the initiation of senescence in the leaves, the cambial zone gradually became narrow with 1–2 layers of cells. The radial seriation and tangential continuity of the cambial zone between secondary xylem and phloem was lost with the differentiation of all cambial cells when the leaves were fully senescent. The study revealed the continuation of primary growth into secondary growth in the petiole with no well-defined transition zone in between.

*) RAJPUT K. S., RAO K. S., Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda – 390 002, India. B. R. D. School of Biosciences, Sardar Patel University, Vallabh Vidyanagar – 388 120. E-mail: ks.rajput15@yahoo.com

## Zusammenfassung

RAJPUT K. S. & RAO K. S. 2011. Development of vascular cambium in the petiole of *Terminalia catappa* L. (*Combretaceae*). [Entwicklung des vaskulären Kambiums im Blattstiel von *Terminalia catappa* L. (*Combretaceae*)]. – Phyton (Horn, Austria) 51 (2): 289–297, mit 2 Abbildungen.

In dieser Arbeit werden Struktur und der Entwicklung des vaskulären Kambiums des Blattstiels von *Terminalia catappa* L. (*Combretaceae*), sowie Muster des sekundären Wachstums histologisch untersucht. Das Kambium des Blattstiels hat nur einen Layer. Die Kambialstrahlen haben vertikal verlängerte fusiforme, sowie kleine isodiametrische Zellen. Nach der Bildung einiger weniger Protoxylem- und Protophloem-Elementen aus dem Prokambium entwickeln sich durch perikline Teilungen im Metakambium das Metaxylem und das Metaphloem. Im Vergleich zum Hauptspross ist die Differenzierung des interfaszikulären Kambiums, ebenso wie das Verbinden des faszikulären Kambiums mit dem interfaszikulären, sehr langsam. Durch eine Zunahme von Metaxylem- und Metaphloemelementen sind im zweiten Blatt die Prokambialstränge ausgeprägter. Der tangentiale Zusammenhang zwischen den Metakambialzellen wird durch die Anlage eines vaszikulären Kambiums zwischen den Bündeln im dritten Blatt auffälliger. Das Kambium bleibt bis zur Seneszenz der Blätter aktiv. Mit Beginn der Seneszenz der Blätter wird die Kambialzone 1–2 Zelllagen schmal. Sind die Blätter ganz gealtert, geht durch die Ausdifferenzierung aller Kambialzellen die radiale reihenweise Anordnung und die tangentiale Kontinuität der Kambialzone zwischen sekundärem Xylem und Phloem verloren. Die Untersuchung zeigt den kontinuierlichen Übergang vom primären zum sekundären Wachstum im Blattstiel mit einer nicht gut definierten Übergangszone dazwischen.

## Introduction

The leaves play vital role in the photosynthesis and are associated with several other complex physiological processes. They are well designed to carryout their functions; usually having broad, flat and thin blades which are connected to the main stem by a stalk called petiole. The substantial amount of water and solutes entering into the leaves and photosynthates produced by the leaves pass through the vasculature of the petiole. In spite of such an important role played by the petiole, the information related to the organization of vascular system in leaves in general and petiole in particular is meager (ISEBRANDS & LARSON 1974, 1977, RUSSIN & EVERT 1984). Therefore, to understand the leaf structure and function, studies on organization and ontogeny of primary vascular tissues in the leaf and petiole are necessary (ISEBRAND & LARSON 1977, LARSON 1982, RUSSIN & EVERT 1984). Cambium of leaf has received little attention, mostly because it has not been widely appreciated that leaves commonly undergo secondary growth. However, a few studies have been made on the leaf cambium of trees growing in temperate regions (EWERS 1982, LARSON 1982, EWERS & ALONI 1987) compared with temperate species, there is virtually no information available on the secondary growth pattern in leaves of tropical and subtropical tree species. Therefore, the present work was undertaken to study the inception, structure and development of vascular cambium and nature of secondary growth in the petiole of *Terminalia catappa* a tropical semi-deciduous tree.

## Material and Methods

Samples of petiole measuring one to two cm long from *Terminalia catappa* depicting different developmental stages from very young to senescent leaves were collected and fixed in Formalin -acetic acid- alcohol (Berlyn & Miksche 1976) (formalin, acetic acid, 70% ethyl alcohol; 10:5:85, v/v) and after 12 hrs of fixation, they were cut into 3–5 mm pieces and transferred into 70% alcohol. Suitably trimmed samples were dehydrated through tertiary butyl series and processed by routine method of paraffin embedding (Johanson 1940). For convenience of comparing the observations, the petioles of different ages have been collected from the youngest leaf on the shoot apex. To study the distribution and extent of the secondary growth, petioles from mature leaves produced in previous growth flush showing progressive age and senescence were also collected. Serial transverse and tangential longitudinal sections of 10–15 μm thick were obtained on a rotary microtome. The sections were stained with safranin-fast green and toluidine blue (Sakai 1973). Petioles of young leaves were examined for understanding the origin of primary vascular tissue and initiation of secondary growth. The mature leaf petioles were studied for the extent and nature of secondary growth. The dimensions of procambium, fusiform cambial cells and their derivatives (primary and secondary xylem elements) were recorded using an ocular micrometer.

## Results

### Development of Vascular Cambium

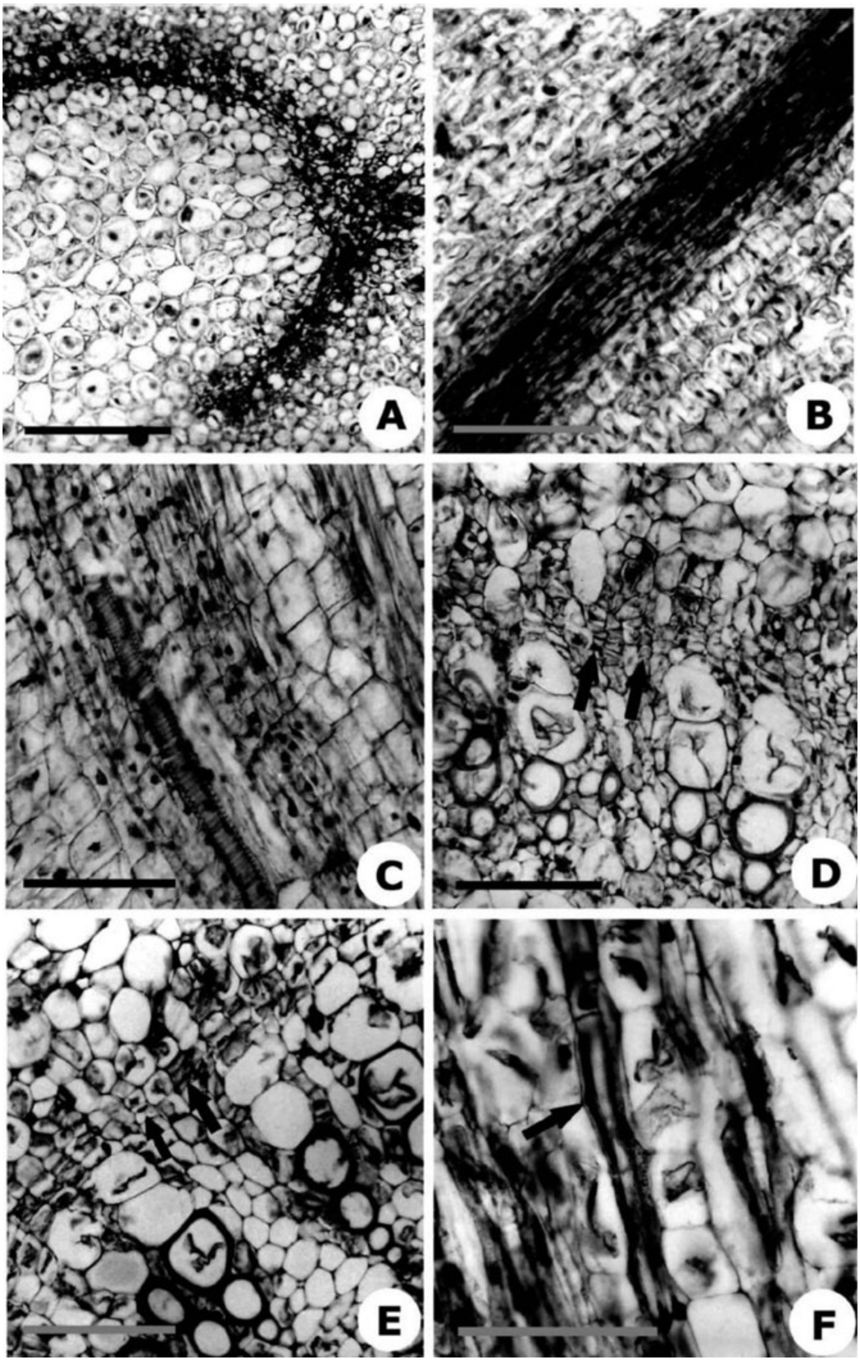
The first differentiating leaf close to the shoot apex had a petiole of 6 mm in length containing about 38 vascular strands forming an arc on adaxial side, leaving a continuous gap between the pith and cortex (Fig. 1A). Each strand was composed of 2–3 layers of procambial cells with protophloem and protoxylem elements on the either side. Procambial cells were axially elongated, thin walled, uninucleate, elongated with narrow lumen (Fig. 1B). Length and width of these cells measured from 23.5 μm to 39.0 μm and 3 μm to 6 μm respectively. A single secretary duct was noticed in the pith region close to protoxylem elements in the central part of each petiole. The petiole of the second differentiating leaf from the shoot apex measured about 9 mm long and showed increased number of vascular bundles with 2–3 thin walled differentiating metaxylem elements. At this stage, protoxylem elements are more distinct along the vascular strands and their number increased in each radial file (Fig. 1C). The cambium giving rise to these elements was considered as metacambium (Larson 1982). Each vascular bundle was separated from adjacent bundle by 2–3 radial rows of interfascicular parenchyma cells (Fig. 1D). In the third leaf, metacambial cells were organized more distinctly and arranged in radial rows of 3–4 layers with differentiating xylem elements. The interfascicular parenchyma cells underwent periclinal divisions resulting in the inception of interfascicular cambium forming a continuous ring of cambial cells between the pith and cortex (Fig. 1E). At this stage, the length and width of metacambial cells varied from 37.5 µm to 46.5 µm and 4.5 µm to 7.5 µm respectively. The diameter of metaxylem showed progressive increase form the protoxylem. In the distal region of fourth leaf, vascular structure of petiole was more or less similar to the basal region of third leaf petiole. The length and width of metacambial cells measured about 45 to 82.5 µm and 4.5 to 9 µm respectively. At this stage, the medullary phloem showed distinct sieve tube elements accompanied by well developed companion cells (Fig. 1F). From the fourth leaf, initiation of secondary growth started with the differentiation of secondary xylem and phloem.

## Secondary Growth

In the petioles of 5$^{th}$ and 6$^{th}$ leaf from the shoot apex, metaxylem tracheary elements were completely differentiated showing lignified thick walls. The first elements to differentiate from vascular cambium were the xylem fibres. The short cells developed from the interfascicular region of cambium contributed to the development of xylem and phloem rays (Fig. 2A). The cambium from the petiole of green mature leaf possessed 3–4 layers of cambial cells with 4–5 differentiating xylem elements in each radial file (Fig. 2B, C). The cambium was nonstoried with elongated fusiform cambial cells and short isodiametric ray cambial cells (Fig. 2A, D). Prior to the yellowing of the leaf blade, the cambium became dormant with 2–3 layers of cambial cells (Fig. 2E). The length and width of fusiform cambial cells varied from 124 µm to 154 µm and 15 µm to 18 µm respectively. The cambium of adaxial side of petiole was more active producing 7 to 10 layers of xylem elements in median position of the transection. The secondary xylem was composed of fibres, vessel elements, axial and ray parenchyma cells. The fibres were elongated with lignified thick walls sharing simple

---

Fig. 1. A–F. Transverse (A, D, E) and longitudinal (B, C, F) section of petiole of *Terminalia catappa*. A: A part of the arc-like procambial strand in a young petiole of first leaf from shoot apex. B: Densely stained procambial cells between cortex and pith cells. C: Metacambial cells showing differentiating metaxylem elements followed by distinct protoxylem elements in the second leaf from the shoot apex. D: Differentiation of metaxylem elements from radially arranged metacambial cells and differentiation of interfascicular parenchyma (arrows) in the second leaf. E: Radially enlarged metaxylem elements and differentiation of interfascicular cambium (arrows) in the third leaf. F: Medullary phloem bundle showing two contiguous sieve tubes with associated companion cells in the fourth leaf (arrow). Fig. 1 A–B: Scale Bar = 150 µm, C–E = 100 µm, F = 75 µm.


A
B
C
D
E
F

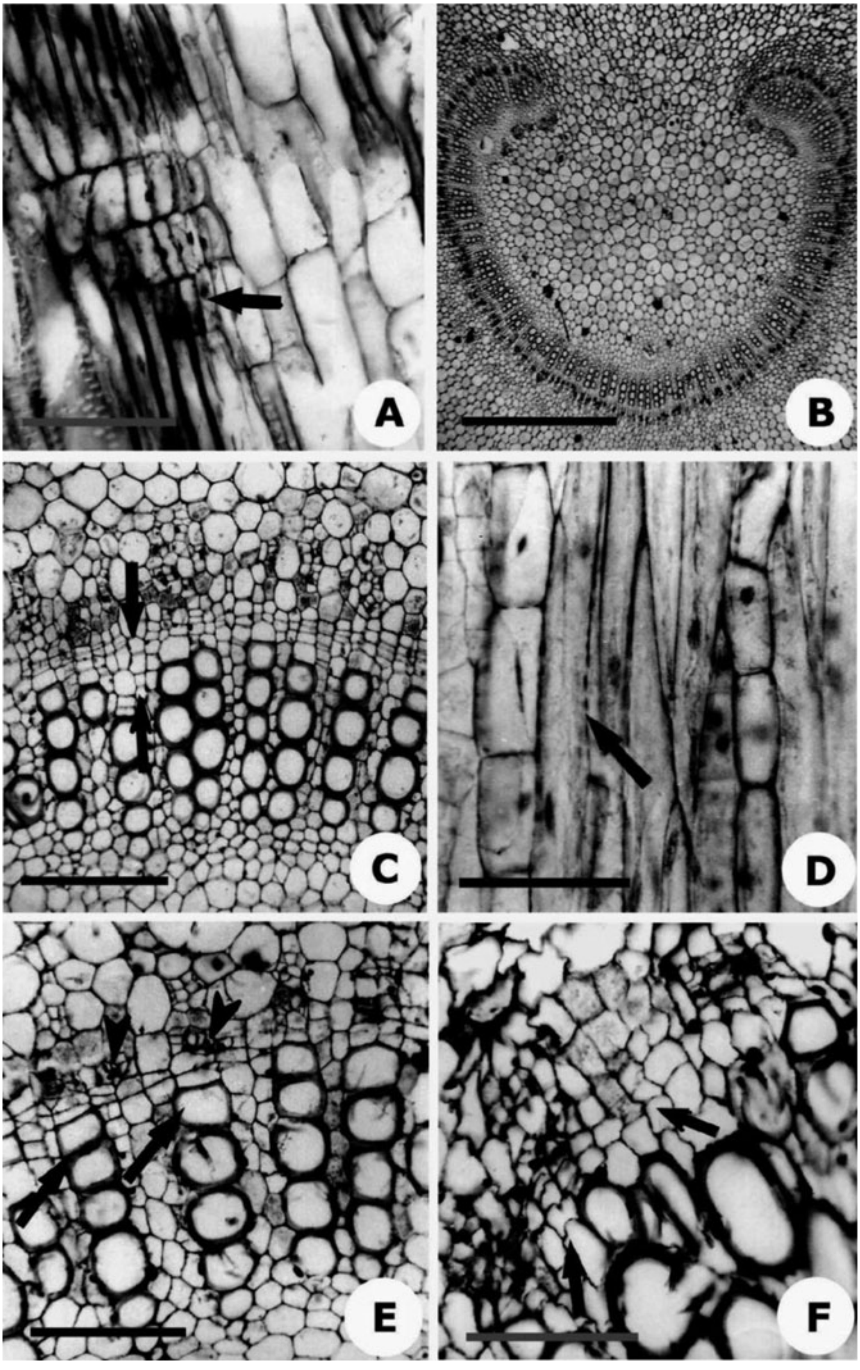
pits. Vessels were solitary and rarely appeared in radial multiples or in groups. Vessels possessed simple, oblique perforation plate and intervessel alternate bordered pits on lateral walls. Axial parenchyma of the xylem were thick walled and elongated while ray parenchyma cells were rectangular with numerous simple pits. In the older leaves gelatinous fibres were distributed among the xylem elements. Each gelatinous fibre possessed a distinct G-layer in close contact with the inner fibre wall. In the petiole of senescent leaf, cambium was 1–2 layered and often not distinct (Fig. 2F). Cambial cells also underwent differentiation that led to the disruption of radial seriation and tangential continuity of cambial zone. The cell arrangement in the phloem was also disturbed due to the obliteration of phloem elements and parenchyma cells. Some of the parenchyma cells in xylem and phloem were filled with phenolics contents. Crystals of calcium oxalate were present in both xylem and phloem ray cells.

## Discussion

As a tropical semi-deciduous tree *Terminalia catappa* L., does not bear leaves all round the year and tree remains leafless for a short period. Usually young leaves sprout immediately following leaf shedding. The petiole of mature leaves has distinct secondary xylem produced from vascular cambium. Secondary growth in the petiole starts with the maturation of leaves. Primary xylem elements are characterized by narrow thick walled protoxylem and wider and angular metaxylem treachery elements. Procambial differentiation and formation of primary conducting elements are similar to those of reported in dicotyledonous stem. The delimitation between metaxylem and secondary xylem as seen in transection of petiole is better defined because secondary growth initiates with the formation of several layers of thick walled radially seriated fibres which appearing distinctly from the contiguous wide primary xylem treachery elements. However, the transition from primary to secondary tissues occurs as a continuation and precise anatomical differentiation of transition does not exist as in stem (Isebrands & Larson 1974).

---

Fig. 2. A–F. Radial (A), transverse (B, C, E, F) and tangential longitudinal (D) section of petiole of *Terminalia catappa*. A: Radial longitudinal view of xylem passing through cambium showing short ray cells in the $6^{th}$ leaf (arrow). B: Basal region of petiole showing complete vasculature in a mature leaf. C: Development of xylem and phloem ray cells from interfascicular cambial cells (arrows) in green mature leaf. D: Cambium showing fusiform initials with beaded radial walls (arrow) and vertically elongated ray initials in a mature leaf. E: Petiole showing rectangular secondary phloem (arrow heads) and differentiating secondary vascular elements (arrows) in mature leaf. F: Cambium from senescent leaf showing differentiating cells towards xylem (arrows). Fig. 2A, E: Scale Bar = 150 μm, B = 1000 μm, C =150 μm, D, F =75 μm.


A
B
C
D
E
F

The pattern of vascular cambium development remains similar to that reported in the stem. In *Terminalia catappa* cambium initiates first at the basal region just above the pulvinous petiole and then extends to the distal region. This results in the gradual decrease in the extent of secondary vascular tissue from the basal to distal region of petiole. However, the cambium may develop first in the middle of petiole and then extends bi-directionally (HOWARD 1974). Although the production of secondary xylem elements is fairly large, the petiole diameter does not undergo significant variation from young to mature leaf. It appears that the secondary xylem elements are accommodated at the expense of thin walled phloem and cortical cells in the petiole. However, secondary growth starts in the petiole when the leaf completely mature. The cambium remains 3–5 layered in all the mature leaves regardless of the age and position on the shoots. The petiole cambium always remains 3–5 layered because the rate of cell differentiation is paralleled by the rate of cell division. It is also interesting to note that the ray initials of petiole cambium give rise to thin walled uniseriate ray cells towards the xylem and phloem. In woody stems, the rate of cambial cell differentiation does not keep pace with the rate of cell division resulting into a distinct seasonal flux in the width of the cambial zone (PHILIPSON & al. 1971, FAHN 1982). In the present study, similar to pine needle cambium, the rate of cell differentiation keeps a pace with cambial cell divisions (EWERS & ALONI 1987). From the present study, it is clearly evident that secondary thickening takes place in the petiole during the leaf maturation in *Terminalia*. However, a comprehensive study of a large number of plants is needed to elucidate the structure, development of and activity of vascular cambium in leaves of tropical and sub-tropical deciduous tree species. The most significant anatomical feature of secondary xylem of petiole is the presence of gelatinous fibres (G-fibres), which are characterized by non-lignified secondary wall layers. G-fibres are extensively studied in the stem and roots of many plants (ZIMMERMANN & BROWN 1974, FISHER & EVERT 1982, RANJANI & KRISHNAMURTHY 1987). However, there are no reports on the occurrence of G-fibres in leaf xylem. Such fibres have also been noticed in the secondary xylem *Mangifera* leaf (unpublished results). Occurrence of G-fibres in mature leaf of *Terminalia catappa* may be associated with the gravitational force exerted on the petiole.

## References


BERLYN G. P. & MIKSCHE J. P. 1976. Botanical Microtechnique and Cytochemistry, pp. 326. – The Iowa State Univ. Press, Ames, Iowa.

EWERS F. W. 1982. Secondary growth in needle leaves of *Pinus longaeva* (Bristle cone pine) and other conifers quantitative data. – Am. J. Bot. 69: 1552–1559.

EWERS F. W. & ALONI R. 1987. Seasonal secondary growth in needle leaves of *Pinus strobus* and *Pinus bruta*. – Am. J. Bot. 74: 980–987.

FAHN A. 1982. Plant anatomy, 3rd ed. – Pergamon Press, Oxford.

FISHER D. G. & EVERT R. F. 1982. Studies on the leaf of *Ameranthus retroflexus*: Morphology and Anatomy. – Am. J. Bot. 69: 1133–1147.

HOWARD R. A. 1974. The stem-node-leaf continuum of the dicotyledons. J. Arnold. – Arbor. Harv. Univ. 55: 125–173.

ISEBRAND J. G. & LARSON P. R. 1974. Anatomy of the primary – secondary transition zone in stems of *Populous deltoids*. – Wood Sci. & Technol. 8: 11: 26.

ISEBRAND J. G. & LARSON P. R. 1977. Organization and ontogeny of the vascular system in the petiole of eastern cottonwood. – Am. J. Bot. 64: 65–77.

JOHANSON D. A. 1940. Plant Microtechnique, pp. 523. – Mc.Graw Hill, New York.

LARSON P. R. 1982. The concept of cambium. – In: BASS P. (Eds.), New perspectives in wood anatomy. – Martinus Nijhott, The Hague.

PHILIPSON W., WARD J. M. & BUTTERFIELD B. G. 1971. The vascular cambium, its development and activity. – Chapman & Hall, London.

RANJANI & KRISHNAMURTHY K. V. 1987. A comparative study of root and stem wood of some members of *Mimosoiae* (Leguminosae). – J. Arnold Arbo. 68: 349–355.

RUSSIN A. W. & EVERT R. F. 1984. The studies on the leaf of *Populous deltoids* (Salicaceae): Morphology and anatomy. – Am. J. Bot. 71: 1398–1415.

SAKAI W. S. 1973. Simple method for differential staining of paraffin embedded plant material using toluidine blue O. – Stain Technol. 48: 247–249.

ZIMMERMANN M. H. & BROWN C. L. 1974. Trees structure and function. – Springer Verlag, Berlin.

# ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2011

Band/Volume: 51_2

Autor(en)/Author(s): Rajput Kishore S., Rao Karumanchi S.

Artikel/Article: Development of Vascular Cambium in the Petiole of Terminalia catappa L. (Combretaceae). 289-297