

Structure and development of cortical bundles in *Couroupita guianensis* Aubl. (Lecythidaceae)

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Estructura y desarrollo de haces corticales en Couroupita guianensis Aubl. (Lecythidaceae)

El desarrollo de haces corticales, en ramas y pedúnculos de *Couroupita guianensis* (Lecythidaceae), comienza cerca del meristemo apical concomitante con los haces vasculares normales. Cada haz cortical llega a estar rodeado por una vaina de fibras que, a menudo, mostraba la presencia de una capa gelatinosa (fibras G). A medida que avanza el crecimiento, cada haz se puede dividir en dos o tres haces. Algunos de los haces son mayores y muestran elementos vasculares bien diferenciados debido a su asociación con frutos en desarrollo, mientras que los más pequeños, con pocos vasos, pueden ser trazas foliares o de yemas de flores que caen antes de la fecundación. El xilema secundario del haz cortical está compuesto de vasos, fibras y células del parénquima axial, mientras que el floema consiste en tubos cribosos, células de acompañamiento y células del parénquima axial.

Palabras clave: Haz cortical, Haz vascular, Traza foliar.

Abstract

The development of cortical bundles, in the branches and peduncles of *Couroupita guianensis* (Lecythidaceae), initiates close to the apical meristem concomitant with the normal vascular bundles. Each cortical bundle becomes surrounded by a sheath of fibres, which most often showed presence of gelatinous layer (G-fibres). As growth progresses, these bundle may divide into two-three bundles. Some of the bundles are larger and show well differentiated vascular elements due to their association with developing fruits while narrower bundles, with few vessels, may be leaf traces or flower bud traces that fell down before fertilization. The secondary xylem of cortical bundle is composed of vessels, fibres and axial parenchyma cells while phloem consists of sieve tubes, companion cells and axial parenchyma cells.

Key words: Cortical bundle, Vascular bundle, Leaf trace.

Introduction

Couroupita guianensis Aubl. (Lecythidaceae) is a remarkable South American tree with large pink and white beautiful flowers, which makes it a favourite avenue tree. It bears, flowers on the main stem as cauliflorous raceme inflorescence, thus the flowers hang from main stem. The fruits reach up to 20-25 cm in diameter and take about 12 to 18 months for maturation and seed dispersal. To cope up with the weight of fruit, peduncles undergo secondary growth and the cambium remains active throughout the year (Rao & Rajput 1996; Rajput *et al.* 2014). During our study on seasonal behaviour of vascular cambium in *C. guianensis* we have noticed presence of cortical bundles in both branches and peduncles.

In addition to normal vascular bundles, certain plants show presence of vascular bundles in the cortex. According to Metcalfe & Chalk (1983), leaf trace bundles that connect the vascular system of the leaf to that of the stem that run longitudinally in the cortex for some distance before their lower ends unite with the main vascular system. In contrast, Karrfalt (1975) considered them as additional bundles other than leaf traces existing in the cortex. There are about 47 dicot families that show existence of cortical bundles including the Lecythidaceae (Metcalfe & Chalk 1950). Their restricted distribution only in certain families may be used as an important taxonomic character but it is not always necessary that they will occur in all the members of the particular family (Majumdar 1941). According to Mauseth (2006) cortical bundles might have evolved through mutation that permitted cortical cells to express the genes ectopically which usually express in leaves.

Though, the occurrence of such bundles is reported in several genera, it is poorly represented in the literature due to lack of detailed information. However, occurrence of such leaf traces (vascular bundles) in the branches is reported in the members of Lecythidaceae (Metcalfe & Chalk 1983) but no information is available on their occurrence in the peduncles. Therefore, the present investigation is aimed to elucidate the structure and development of cortical bundles in peduncle of *C. guianensis*.

Material and methods

Samples of young and mature peduncles and branches measuring from 5-20 mm thickness were collected from the *C. guianensis* growing in the University Botanical Garden and Arboretum (22° 19' 15.9" N and 73° 10' 45.1" E at an altitude of 35.5 m). To study the development stages of cortical bundles, samples from apical portion up to 15 nodes were collected from peduncles and actively growing branches. Details of the sampling and selection of trees is described elsewhere (Rajput *et al.* 2014). Immediately after collection, samples were fixed in FAA (Berlyn & Miksche 1976). After 24 hrs of fixation, they were transferred in 70 % alcohol for storage and further processing.

Samples were trimmed into 5x5 mm pieces and processed for paraffin embedding as described by Johansen (1940). Serial sections of peduncles and branches were obtained with the help of Leica rotary microtome to study the developmental stages of cortical bundles. Paraffin embedded samples were cut into 15-20 µm thick sections in transverse, radial and tangential planes and stained with safranin-fast green combination (Johansen 1940). After dehydration through ethanol-xylene series, they were mounted in DPX (Dibutylphthalate polystyrene xylene). Important results were micro-photographed with Leica trinocular research microscope. Fully grown peduncles were collected and kept in water for retting, to remove the bark. After retting, the xylem portion of the cortical bundles was used for maceration process. Three to four mm long pieces of the material was kept in Jeffrey's solution (Berlyn & Miksche 1976) at 55 to 60 °C for 24-36 hrs and stained with aqueous safranin to study general morphology and size of vessel elements and fibres. Thirty measurements for fibres and vessel elements were measured randomly to obtain their length.

Results

Anatomy of young branches and peduncles

In young branches and peduncles, a single cell layered epidermis enclosed several cells wide cortex (Fig. 1A). Cortex was composed of thin

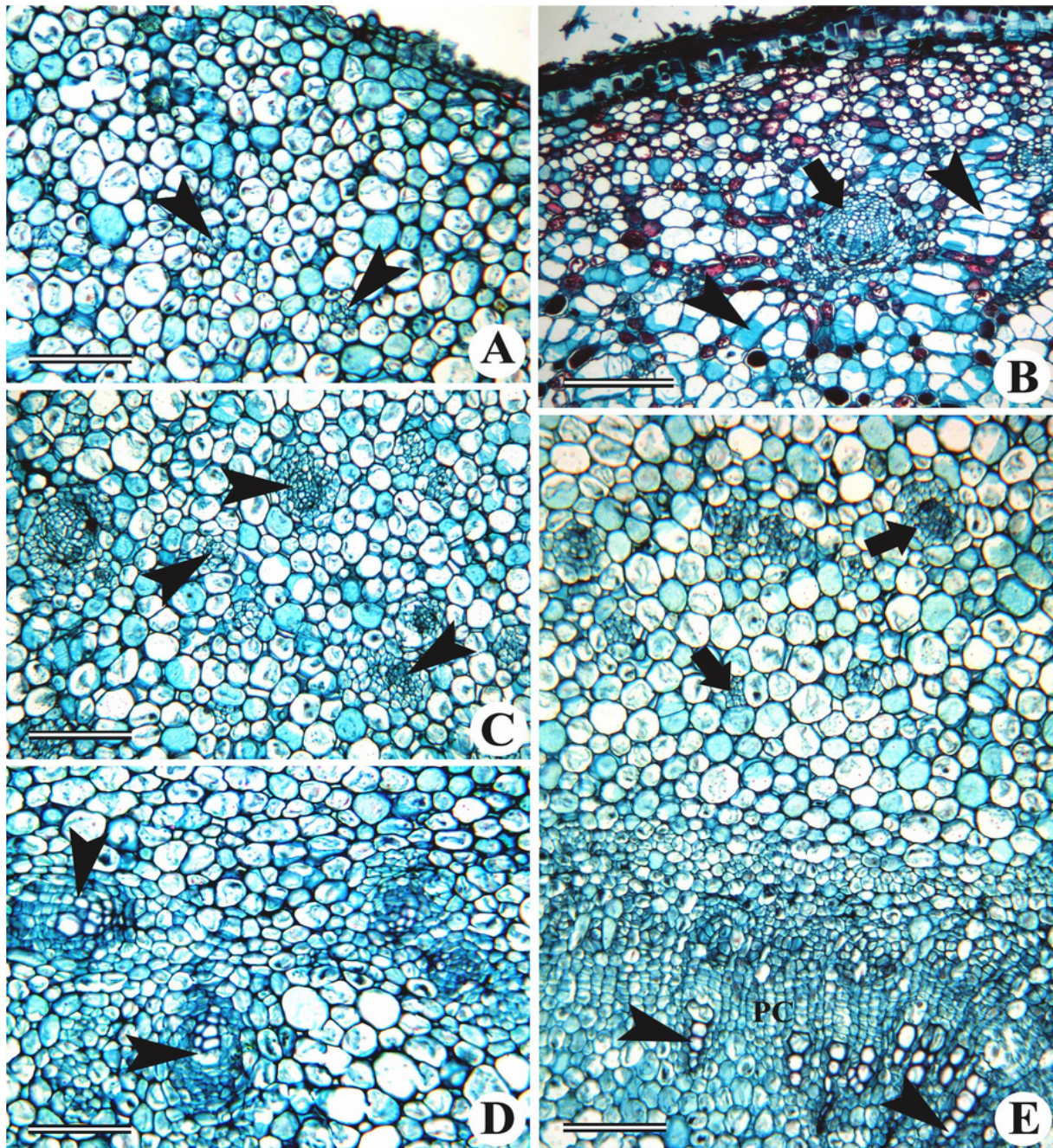


Figura 1. Cortes transversales de pedúnculo (A, C-E) y rama (B) de *Couroupita guianensis* mostrando diferentes estados de desarrollo de los haces corticales. **A:** Pedúnculo joven mostrando una corteza ancha compuesta por parénquima oval-redondeado. Las puntas de flechas indican el inicio de la diferenciación del haz cortical; **B:** Parénquima cortical adyacente al haz cortical (flecha) mostrando el alargamiento radial y tangencial (puntas de flecha); **C:** Corteza con algunos haces corticales mostrando varios estados de desarrollo (cabezas de flecha); **D:** Orientación irregular de haces corticales (cabezas de flecha). Véase la orientación del xilema y floema; **E:** Origen simultáneo de los haces vasculares a lo largo del procambium (PC) y protoxilema (cabezas de flecha). Las flechas indican haces corticales. Barras de escala: C-E= 125 μ m; B= 250 μ m.

Figure 1. Transverse views of peduncle (A, C-E) and branch (B) of *Couroupita guianensis* showing different stages of cortical bundle development. **A:** Young peduncle showing wide cortex composed of oval to round shaped parenchyma. Arrowheads show initiation of cortical bundle differentiation; **B:** Cortical parenchyma adjacent to cortical bundle showing radial and tangential enlargement (arrowheads). Arrows indicate cortical bundles; **C:** Cortex with several cortical bundles showing various developmental stages (arrowheads); **D:** Irregular orientation of cortical bundles (arrowheads). Note the orientation of xylem and phloem. **E:** Simultaneous origin of cortical bundles along with the procambium (PC) and protoxylem (arrowheads). Arrows indicate cortical bundles. Scale bars: C-E= 125 μ m; B= 250 μ m.

walled parenchyma cells and differentiating cortical bundles. However, as the growth progressed further, the cells in the middle part of cortex underwent radial and tangential expansion and makes the outer cortex distinct (Fig. 1B). The cortical region was comprised of several cortical bundles of various sizes distributed randomly in the cortical region (Fig. 1C, 1D). Several collateral vascular bundles joined by interfascicular cambium forming a complete ring. Vascular cambium was visible at the 5th internode (Fig. 1E). Functionally, the cambium was bidirectional in both branches and peduncles producing secondary phloem centrifugally and secondary xylem centripetally.

Structure of cortical bundles

Cortical bundles were initiated close to the apical meristem concomitant with the normal vascular bundles. As shown in figures 1D and 1E, the number of xylem and phloem elements in normal vascular bundles and cortical bundles observed was more or less similar. In the beginning of their development cortical bundles were closely spaced (Figs. 1C, 1E, 2C) but with the increase in thickness of peduncle, the cortex expanded thus making the cortical bundles widely spaced (Fig. 2A, 2B). At the initial stage of development, they are the part of the main vascular bundles which gradually deviated from the vascular cylinder toward the cortex (Fig. 2D-F). These bundles were variously oriented with different sizes often showing quantifiable amount of xylem and phloem elements (Figs. 2F, 3A). Such large cortical bundles are observed only in peduncles while cortical bundles in the branches possessed limited number of xylem elements. In relatively thick branches and peduncles, each bundle was encircled by fibres, which often showed G-layer (Fig. 3A). In contrast, some of the cortical bundles remained smaller in size probably due to cessation of further growth or shedding the flower buds. It is evident from the serial transverse section (Fig. 3B-D) that the bundles from each other apart merge together which indicates that they undergo branching or form anastomosing network. Sometimes, union of more than two such cortical bundles was also noticed in peduncles (Fig. 3E).

Structurally, secondary xylem of cortical bundles was composed of vessel elements, tracheids and axial parenchyma cells. As com-

pared to normal secondary xylem, tracheary elements of cortical bundles were narrower and mostly consisted of vessels while fibres and parenchyma cells were relatively less. Vessels may be solitary or arranged in radial multiples with helical thickening in earlier formed vessels and alternate bordered pits on later formed vessels of large cortical bundles. The length and width of the vessel elements ranged from 295 μm to 337 μm in and 60 μm to 128 μm respectively. The length of fibres varied from 839 μm to 946 μm . Secondary phloem was composed of sieve tube elements, companion cells, axial parenchyma and uniseriate rays. The sieve tube elements measured from 278 μm to 295 μm in length.

Discussion

Depending on the phyllotaxy of plant, one or more number of vascular bundles from each node diverges from the main vascular cylinder and provides vascular supply to the leaf/leaves originating from that node. Such supply of vascular bundle from the main vascular cylinder to leaf is called as leaf trace (Evert 2006). However, in some plants these vascular bundles extend for great longitudinal distance before they enter in leaves are referred as cortical bundles (Beck 2010). Their occurrence is limited to 47 families of dicots and has taxonomic significance due to their restricted distribution (Metcalf & Chalk 1983). Presence of cortical bundles is characteristic to Lecythidaceae and its occurrence in the stem of *Couroupita* Aubl. is reported by Metcalfe and Chalk (1950). Although its occurrence is documented, no detailed information is available on its course of development. In the present study we report presence and development of cortical bundles in peduncles and branches of *C. guianensis*.

Development of cortical bundles occurs simultaneously with the origin of procambium and protoxylem in the peduncles and branches of vascular cambium. During their development some of the cells in the cortex divide and differentiate into cortical bundles. The first differentiating elements are always sieve tube elements while protoxylem production occurs subsequently. First development of phloem elements may be associated with photosynthate supply to the developing leaves and floral buds in the branch and inflorescence respectively. As the peduncle or branch grows fur-

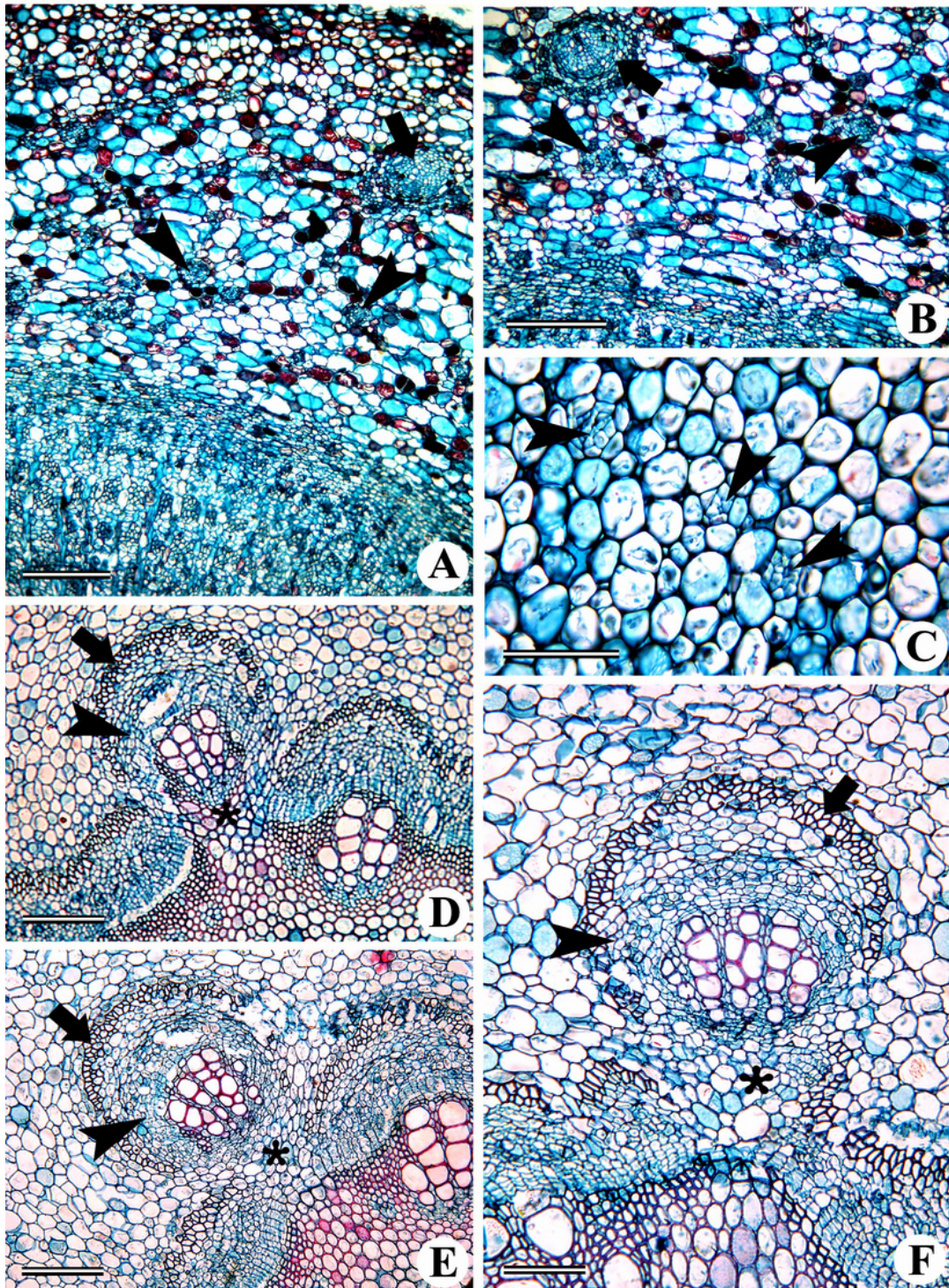


Figura 2. Cortes transversales de pedúnculo (A, C-E) y rama (B) de *Couroupita guianensis* mostrando diferentes estados de desarrollo de los haces corticales. **A:** Hazes corticales muy separados en rama delgada. Véase que sólo unos pocos haces corticales tienen un cuantificable número de elementos del xilema y floema (flecha); **B:** Hazes corticales muy separados en pedúnculo delgado (puntas de flecha); **C:** Hazes corticales estrechamente espaciados en un pedúnculo joven (puntas de flecha); **D-F:** Separación del haz cortical (punta de flecha) del principal cilindro vascular. Véase el aumento de la distancia y las células parenquimáticas en la cara interna del haz (*). Las flechas indican el anillo de fibras que rodean al cilindro vascular. Barras de escala: A-B= 250 μ m; C-F= 75 μ m.

Figure 2. Transverse view of branch (A) and peduncle (B-F) of *Couroupita guianensis* showing different stages of cortical bundle development. **A:** Widely separated cortical bundles in a thick branch. Note that only few cortical bundles have quantifiable number of xylem and phloem element (arrow). Arrowheads show small vascular bundles; **B:** Widely spaced cortical bundles in a thick peduncle (arrowheads); **C:** Closely spaced cortical bundles in a young peduncle (arrowheads); **D-F:** Separation of cortical bundle (arrowhead) from the main vascular cylinder. Note the increasing distance and parenchyma cells on the inner side of bundle (*). Arrows indicate the ring of fibres encircling the cortical bundle. Scale bars: A-B= 250 μ m; C-F= 75 μ m.

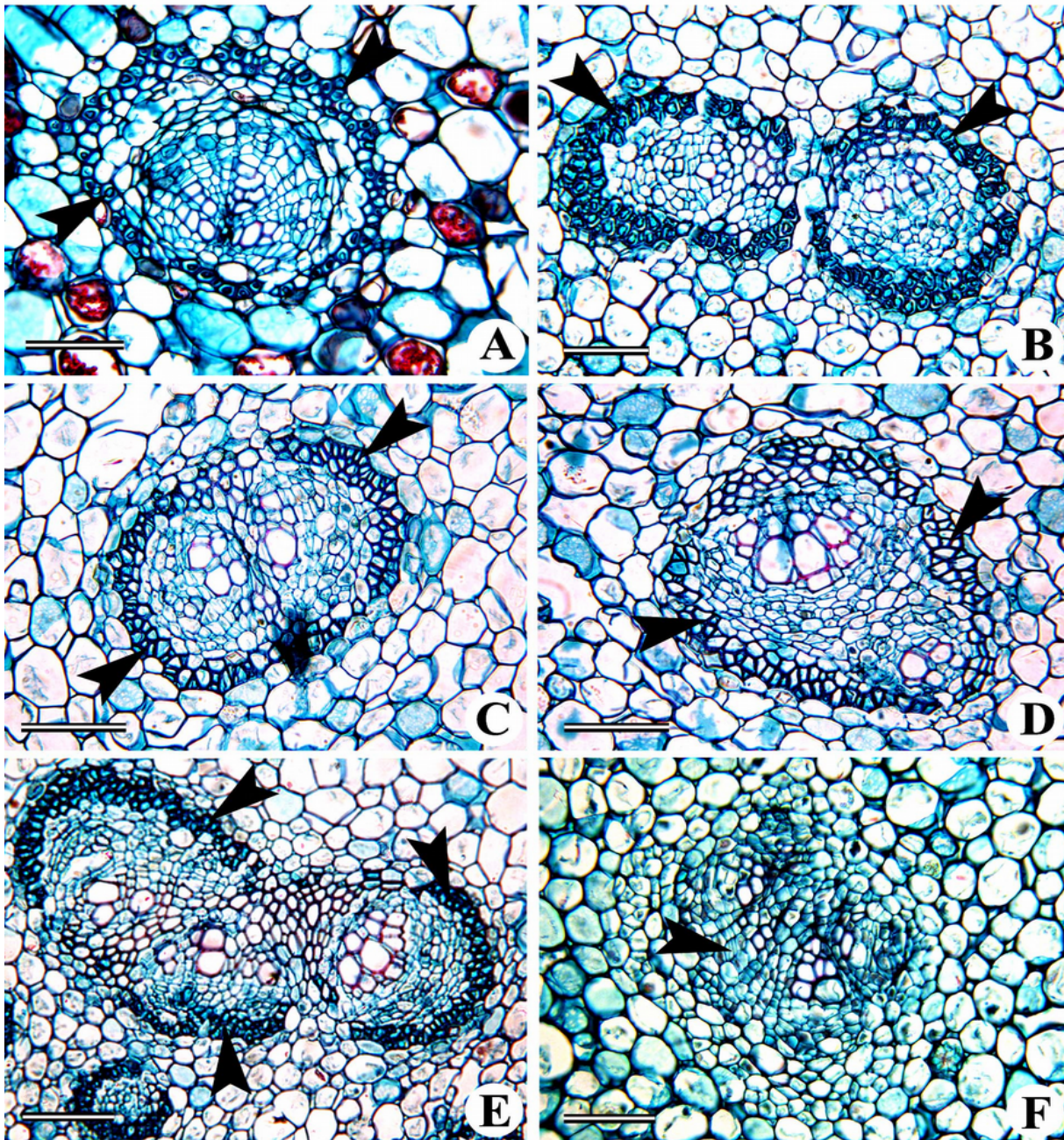


Figura 3. Cortes transversales de pedúnculo mostrando varias orientaciones de los haces vasculares en *Couroupita guianensis*. **A:** Un haz cortical mostrando un anillo de fibras con capa G; **B-D:** Hazes corticales ampliamente separados mostrando su fusión en una serie de secciones transversales; **E:** Uno de los haces mostrando la fusión de tres haces corticales (puntas de flecha); **F:** Un haz mostrando el inicio del procambium (punta de flecha). Barras de escala: 3A-B= 50 μ m; C-F= 75 μ m.

Figure 3. Transverse views of peduncle showing variously oriented cortical bundles in *Couroupita guianensis*. **A:** A cortical bundle showing a ring of fibres with G-layer; **B-D:** Widely separated cortical bundles showing their fusion in serial transverse sections (arrowheads). Note the sequential stages; **E:** One of the bundles showing fusion of three cortical bundles (arrowheads); **F:** One of the bundles showing initiation of procambium (arrowhead). Scale bars: 3A-B= 50 μ m; C-F= 75 μ m.

ther a complete ring of cambium encircles the xylem and form conjoint collateral or bicollateral cortical bundle. These bundles in the cortex appears as circular, dumbbell shaped or inversely oriented. Majumdar (1941) reported inversely orientated cortical bundles in *Nyctanthes arbor-tristis* L.. According to him, these cortical bundles

never directly connected with the main axial ring. Similar results are also documented in *Calycanthus* L. (c.f. Mujumdar 1941). However, in *Couroupita* these cortical bundles are the part of the main vascular system which deviate gradually and remain the cortex.

Karrfalt (1975) studied nature of cortical

bundles in *Adenocaulon lyratum* S. F. Blake (Compositae) and reported their stipular origin. Occurrence of several bundles in the cortex of the young branch and peduncles of *Couroupita* seems to be associated with the spirally arranged leaves on branch and peduncles. Presence of large sized bundles with a distinct xylem and phloem in cortex of peduncles indicates their role in supply of photosynthate to the developing fruits while other bundles with limited number of xylem and phloem elements in the branches suggest their water and food conduction role in leaves. Development of cambium and production of abundant secondary xylem and phloem has also been reported by earlier workers (Mauseth & Sajeve 1992; Terrazas & Mauseth. 2002; Terrazas & Arias 2003; Mauseth 2006). However, it is considered that they are not important in long distance longitudinal transport since the stem has larger, more numerous conducting tissues and if the stem is severely damaged the cortical bundles would not be adequate to conduct the food material (Mauseth & Sajeve, 1992). In *Couroupita* peduncle they may be performing dual function i.e. supply of photosynthate, water and mineral nutrients from peduncle to developing fruits and subsequently mechanical support to them. Mauseth (2006) ascribed similar function of cortical bundles in Cactaceae that the secondary phloem of main vascular cylinder is quite away from the photosynthetic cortex; therefore, all sugars loading must be occurring through the cortical bundle or leaf traces. This hypothesis may not be applicable in the present study but it may be associated with rapid loading and unloading of sugar due to sudden and rapid defoliation. *Couroupita* shows 2-3 times defoliation (December-January, May-June and August) within a year and takes about 8-10 days to replace the whole set of older leaves with new ones (Rajput *et al.* 2014). Therefore, their presence in branches may help in rapid supply of photosynthate from sink to developing leaves and its rapid translocation of sugars from yellowing leaves to stem before defoliation.

Development of vascular cambium followed by secondary growth in cortical bundles is reported in several members of Cactoideae (Mauseth & Sajeve 1992; Terrazas & Mauseth 2002; Mauseth 2006; Terrazas *et al.* 2016; Schwager *et al.* 2015). In the present study also development of secondary xylem and phloem is observed in the cortical

bundles. Terrazas *et al.* (2016) compared structural and dimensional details of tracheary elements of the cortical bundles found in tubercles of *Coryphantha* (Engelm.) Lem. (Cactoidae) with the main vasculature, and reported that the diameter of tracheary elements is threefold narrow than the diameters of those of the vascular cylinders. Such narrow tracheary element is reported in the leaf petioles and branches of dicotyledons and is referred as 'bottleneck' by Zimmermann (1983). A bottleneck was also confirmed by Mauseth & Sajeve (1992) in *Subpilocereus repandus* (L.). In *Couroupita*, the diameter of vessel elements occurring in cortical bundles is almost half as compared to main cylinder of peduncle. The vessel elements of cortical bundles possess simple perforation plate and shorter than those of main vasculature. Similar feature has been reported by Terrazas *et al.* (2016) in six species of *Coryphantha*. Vessel diameter is heterogenous i.e. narrow and wide vessels intermixed or increasing centrifugally or remains constant in different species of *Coryphantha*. In *Couroupita*, vessel diameter increase from the center towards the periphery of cortical bundles.

A ring of fibres encircled the cortical bundles in which many of them often show presence of gelatinous layer (G-layer) in peduncles. In angiosperms, reaction xylem is characterized by occurrence of gelatinous fibres on the upper side of secondary xylem when eccentric growth takes place in stem or branches (Sivan *et al.* 2014). Similar pattern of G-fibre development has also been reported earlier in the peduncles of *Kigelia africana* (Lam.) Benth. (= *Kigelia pinnata* (Jacq.) DC.) (Sivan *et al.* 2010) and in the stems of poplar (Berlyn 1961), *Eucalyptus* L'Hér. (Wahusen *et al.* 2002) and *Quercus* L. (Burkat & Canocapri 1974). G-fibres in the cortical bundles of peduncle may be associated with providing tensile strength to the peduncle to tolerate the weight of the large and heavy fruits. As the fruits increase in size, their increasing weight may be generating higher stress on the peduncle. Fang *et al.* (2007) concluded that tension wood experiences higher growth stress and tensile stress generation is the function of G-layer, which is considered to be indicator of tension wood severity (Sivan *et al.* 2014). Therefore, occurrence of G-fibres in the peduncles may be associated with stress generated by larger fruits of *Couroupita*.

In conclusion, the initiation of cortical bundles in the *Couroupita* starts concomitant with procambium and protoxylem. During their differentiation, protoxylem differentiates prior to protoxylem. Their presence in peduncles indicates its mechanical value to support the large number of big and heavy fruits on single peduncle. In contrast, in branches they may be facilitating rapid translocation of photosynthates. Stress generated by fruits may be responsible for the development of G-fibres in peduncles which may be facilitating to increase the tensile stress to tolerate the weight of the fruits in *C. guianensis*.

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