

COMPARATIVE FOLIAR MORPHOLOGY AND ANATOMY OF *LAGERSTROEMIA* L. (LYTHRACEAE) AND ITS TAXONOMIC IMPLICATIONS

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ABSTRACT

The two introduced species of *Lagerstroemia* in Nigeria are differentiated with some anatomical characters of the leaf and stem using light microscopy. Generic constant features are hypostomatic leaf, anomocytic stomatal type, bicollateral vascular bundle, presence of striations and a ring of sclerenchymatous hypoderm which subtends the vascular bundles. Copious distribution of striations on both surfaces of the leaf epidermis, closed vascular bundles, presence of mucilagenous cells and 25% stomatal index distinguish *L. indica* from *L. speciosa* which has scanty striations that are restricted to the adaxial surface of the leaf epidermis, closed or open vascular bundles, absence of mucilagenous cells and 20% stomatal index. Perhaps, these characters may be confined to individuals from tropical Africa. A bracketed identification key is also presented.

INTRODUCTION

Lagerstroemia L. belongs to the family Lythraceae. It is a genus of about 50 species of deciduous and evergreen trees and shrubs native to the Indian subcontinent, southeast Asia, northern Australia and parts of Oceania; but it has been introduced to the warmer climates around the world including Africa (Little *et al.*, 2004; Mabberley and David, 2008). The genus has two aesthetically important species in Nigeria; they contribute to attractive scenery in most cities and households across the country. The two species are *Lagerstroemia indica* (L.) Pers. and *Lagerstroemia speciosa* (L.) Pers. Apart from the aesthetic value, the species are also economically and medicinally useful. The timber of *L. speciosa* is useful for making bridges, furniture and railway sleepers. The species can be used for relieving ailments such as oedema, diabetes, urinary dysfunction, fevers and digestive disorders. It has also been reported that the plant can control cholesterol, blood pressure and weight loss. Moreover, they can help to control erosion in poorly drained and drought-prone areas.

There is dearth of information on the anatomical characters of the two species. There are also divergent reports on stomata distribution in *L. speciosa* (Anil and Vaikos, 2012; Woratouch *et al.*, 2011). In addition, the possession of hypoderm has not been documented for all species in the family but some taxa of particular origin (Baas, 1986; Solereder, 1908). Therefore, the focus of the study was to provide anatomical data that will assist in species identification and to present the peculiar features of the species growing in Nigeria since environmental factors have been implicated on the anatomy of some of the species.

Moreover, whether hypoderm will be found in our species is another problem the work has endeavoured to address. The data may also be helpful to pharmacognostic research.

MATERIALS and METHODS

Leaf and stem were used for the study. Materials were obtained mainly from many places in the southwestern Nigeria and samples were cross-checked with specimens at the Forestry Herbarium Ibadan (FHI) and Lagos University Herbarium (LUH) for authentication. Anatomy follows the methods of Kadiri *et al.* (2007), Kadiri

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and Ayodele (2010) and Ajayi *et al.* (2011) with some modifications. The terminologies of Wilkinson (1989) were adopted. The stem, petiole and midrib samples were freely sectioned using razor blade and the thin slices obtained were kept in water before transferring onto a glass slide where a few drops of 99% ethyl alcohol were added for tissue hardening and then 2-3 drops of Safranin solution. Excess stain was washed off with water before a drop of glycerine was added. Slides were covered with cover slips and then ringed with nail lacquer to prevent dehydration. Leaf epidermis was examined after Nitric acid treatment which involved cutting 2-5 cm² of the standard median part of the leaf lamina near the mid-rib following the methods of Olowokudejo (1993), Ogundipe and Wujek (2004), Ogundipe and Kadiri (2013) with some modifications. Dried leaves were boiled in water for thirty minutes and subsequently soaked in concentrated trioxonitrate (v) acid (HNO₃) in capped specimen bottles for about 8-24 hrs to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into Petri dishes containing water for cleansing. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. They were passed through a series of ethanol ranging from 50%-100% drops of different grades of ethanol, 50 – 100% to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five minutes before mounting in glycerine on glass slides. The epidermal layers were mounted on glass slides with the adaxial and abaxial surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration. All preparations were observed with an Olympus microscope and photographs were taken with digitized camera for microscope. Line drawings were obtained with the aid of camera lucida drawing apparatus.

RESULTS

A summary of the results is presented in Table 1 and Figs. 1 -3.

LEAF EPIDERMIS

The leaf is generally hypostomatic. On the adaxial surface, the anticlinal wall is straight and mucilagenous cells are present in the epidermis. There are striations on both surfaces of the epidermis of *L. indica* (fig. 1A, B) whereas they are restricted to the adaxial surface in *L. speciosa* (Fig. 1C). On the abaxial surface, the anticlinal wall is curved in both species (Fig. 1B, D) and solitary crystals were found on the adaxial surface of *L. speciosa* (Fig. 1C). There are usually more epidermal cells on the adaxial surface than abaxial surface and cells are generally larger in the latter than the former but stomatal size is almost uniform in the two species (Table 1). Stomatal index varies from 20-25.5%.

MIDRIB

The midrib is dome-shaped in both species on the adaxial surface and unevenly crescentiform on the abaxial surface (Fig. 2G, H). The perivascular bundle is composed of oval shaped cells in *L. indica* or sometimes polyhedral in *L. speciosa*. Crystals of calcium oxalate are present in *L. indica* (Fig. 1H) but they are absent in *L. speciosa* (Fig. 1F). The vascular bundle is centrally located in both species and it is crescentiform in *L. indica* but roughly circular in *L. speciosa* (Fig. 3G, H).

PETIOLE

In *L. indica*, the perivascular cells are usually oval in shape. The parenchyma cells are polyhedral to circular in shape and they are usually filled with chemical substances (Fig. 2A-D). On the adaxial surface, the petiole is deeply furrowed at the proximal position, slightly furrowed at the median and dome-shaped (Fig. 3A-C) at the distal area. On the abaxial surface, it is perfectly U-shaped at both proximal and median positions but deeply U-shaped at the distal end. The vascular bundle is centrally located, bicollateral and surrounded by a

continuous tissue of sclerenchyma (Fig. 3A-C). In *L. speciosa*, the parenchyma cells have irregular shapes, the vascular bundle is collateral and there are cell inclusions (Fig. 2C, D). On the adaxial surface, the outline is uneven at the proximal end, knobbed at the median and dome-shaped at the distal position (Fig. 3D-F). In addition, the vascular bundle is open at proximal and median positions but it is closed at the distal end. It is centrally located, bicollateral and subtended by a continuous sheet of sclerenchyma cells.

STEM

The epidermis of the stem is one-layered, followed by the cortical region that is composed of two layers of collenchyma and sclerenchyma cells each; their shape varies from polyhedral to circular. At the pith region, the parenchyma cells are also polyhedral in shape and sometimes appear globose in the two species (Fig. 2E-H). There are also some solitary crystals, which are more in *L. indica* than *L. speciosa* (Fig. 2F, H).

Table 1: Anatomical characteristics of the leaf and stem of two species of *Lagerstroemia* in Nigeria

SN	Characters	Surface	<i>L. indica</i>	<i>L. speciosa</i>
1.	Leaf epidermal cell shape	Adaxial	Polygonal	Polygonal
		Abaxial	Irregular	Irregular
2.	Anticlinal wall pattern of leaf epidermis	Adaxial	Straight	Straight
		Abaxial	Curved	Curved
3.	Stomatal type on leaf	Adaxial	-	-
		Abaxial	Anomocytic	Anomocytic
4.	Leaf midrib shape	Adaxial	Domed	Domed
		Abaxial	Imperfect crescentiform	Imperfect crescentiform
5.	Petiole	Adaxial	Proximal: Furrowed Median: Imperfectly furrowed Distal: Raised	Proximal: Uneven Median: Knobbed Distal: Dome
		Abaxial	Proximal: Perfectly U-shaped Median: Perfectly U-shaped Distal: V-shaped	Proximal: Crescentiform Median: Crescentiform Distal: Semicircular
6.	Leaf epidermal cell number	Adaxial	97(110±3)123	115(128±4)140
		Abaxial	60(82±5)103	80(103±5)126
7.	Leaf epidermal cell length (µm)	Adaxial	25.3(29.7±2.8)34.0	22.5(33.8±2.4)45.0
		Abaxial	16.0(18.2±0.4)20.3	30.0(34.9±1.2)39.8
8.	Leaf epidermal cell width (µm)	Adaxial	16.7(20.4±0.7)24.6	17.2(26.1±1.1)35.0
		Abaxial	12.0(14.6±0.5)17.2	17.8(24.1±1.3)30.4
9.	Leaf epidermal cell wall thickness (µm)	Adaxial	2.0(2.5±0.1)3.0	0.5(1.8±0.1)2.0
		Abaxial	0.5(1.0±0.2)1.5	1.0(1.5±0.1)2.0
10.	Leaf stomatal number	Adaxial	-	-
		Abaxial	18(28±2)39	12(25±2)38
11.	Leaf stomatal length (µm)	Adaxial	-	-
		Abaxial	16.2(18.0±0.4)19.7	17.0(18.6±0.4)20.1
12.	Leaf stomatal width (µm)	Adaxial	-	-
		Abaxial	12.8(13.2±0.1)13.5	11.5(12.0±0.1)12.5
13.	Leaf stomata index	Adaxial	-	-
		Abaxial	25.5%	20%
14.	Special feature	Adaxial	Mucilaginous cells present	Absent
		Abaxial	Mucilaginous cells around stomata	Absent

DISCUSSION

The anatomical account of the family Lythraceae by Metcalfe and Chalk (1950, 1979) reported on unspecified species of *Lagerstroemia* and the data on them are sparse. Most of these data were confirmed while new ones are now added. Bicollateral vascular bundle, ranunculaceous or anomocytic stomata, unicellular or bicellular trichomes, presence of mucilaginous cells, solitary, slightly curved, crescent-shaped vascular strands and abundance of solitary crystals were some of the features recorded for the genus. Narrow vessel element has also been reported in the family (Baas, 1986). Solereder (1908) also reported a somewhat collenchymatous hypoderm consisting of one layer that is developed on the upper side of the leaf of a genus in the family. Most of these features were found in *L. indica* but *L. speciosa* can be differentiated with the absence of mucilaginous cells, possession of open vascular bundles, presence of striations only on the adaxial surface of the epidermis. Although trichomes were reported by Metcalfe and Chalk (1950, 1979) in the genus using species that were probably not of tropical origin, the two species studied in the present work were glabrous. Stace (1965), Inamdhar and Gangadhara (1977) have noted that trichome distribution can be under the influence of environmental factors. To a reasonable extent, the two species can be differentiated based on these few anatomical characters. *L. indica* has furrowed to raised adaxial surface of the petiole, less than 90 epidermal cells on the abaxial surface, presence of striations on both surfaces of the leaf epidermis, epidermal cell thickness on the adaxial surface that is as much as 2.5 μm and 25.5% stomatal index. Conversely, *L. speciosa* has even or knobbed to dome-shaped adaxial surface of the petiole, more than 100 epidermal cells on the abaxial surface, presence of striations on the adaxial surface only, thin epidermal cell wall that is less than 2.0 μm and 20% stomatal index. Anil and Vaikos (2012) reported amphistomata in *L. speciosa* from India and hypostomata were reported for Thailand representative (Woratouch, 2011). Another important tissue of ecological significance that was recorded in the species is a hypoderm which Anil and Vaikos (2012) reported its development and occurrence as a means to ecological adaptation. Hypoderm is that part of the fundamental tissue in plants immediately beneath the epidermis. It is very frequently thickened so as to add to the mechanical strength of the structure in petioles, peduncles, and herbaceous stem-structures usually of collenchymatous origin.

The genus and species are described in the following taxonomic notes and an identification key is presented to assist in delimiting the species.

Taxonomic Notes

Generic Description

Leaf hypostomatic. Stomatal type anomocytic, Anticlinal wall pattern on adaxial surface usually straight. Stomatal index 20%-25.5%. Vascular bundle centrally located, bicollateral and subtended by a continuous sclerenchyma ring. Mucilaginous cells may be present or absent. Distribution of crystals of calcium oxalate within epidermal cells and parenchyma cells of petiole, midrib and stem.

A bracketed key for species delimitation based on the few anatomical features:

1. Striations present on abaxial and adaxial surfaces of leaf, anticlinal wall undulate on abaxial surface, solitary crystals present on abaxial surface of leaf. Stomatal index 25.5%. Petiole adaxial surface furrowed to dome-shaped. Petiole vascular bundle usually closed. Solitary crystals of calcium oxalate present in midrib. Xylem vessels generally narrow. Mucilaginous cells present*Lagerstroemia indica*
1. Striations present on adaxial surface of leaf only, anticlinal wall curved on abaxial surface, solitary crystals present on adaxial surface of leaf, stomatal index 20%. Petiole curvature uneven or knobbed to dome-shaped on adaxial surface. Petiole vascular bundle open or closed. Solitary crystals of calcium oxalate absent in midrib. Xylem vessels wide. Mucilaginous cells absent.....*Lagerstroemia speciosa*

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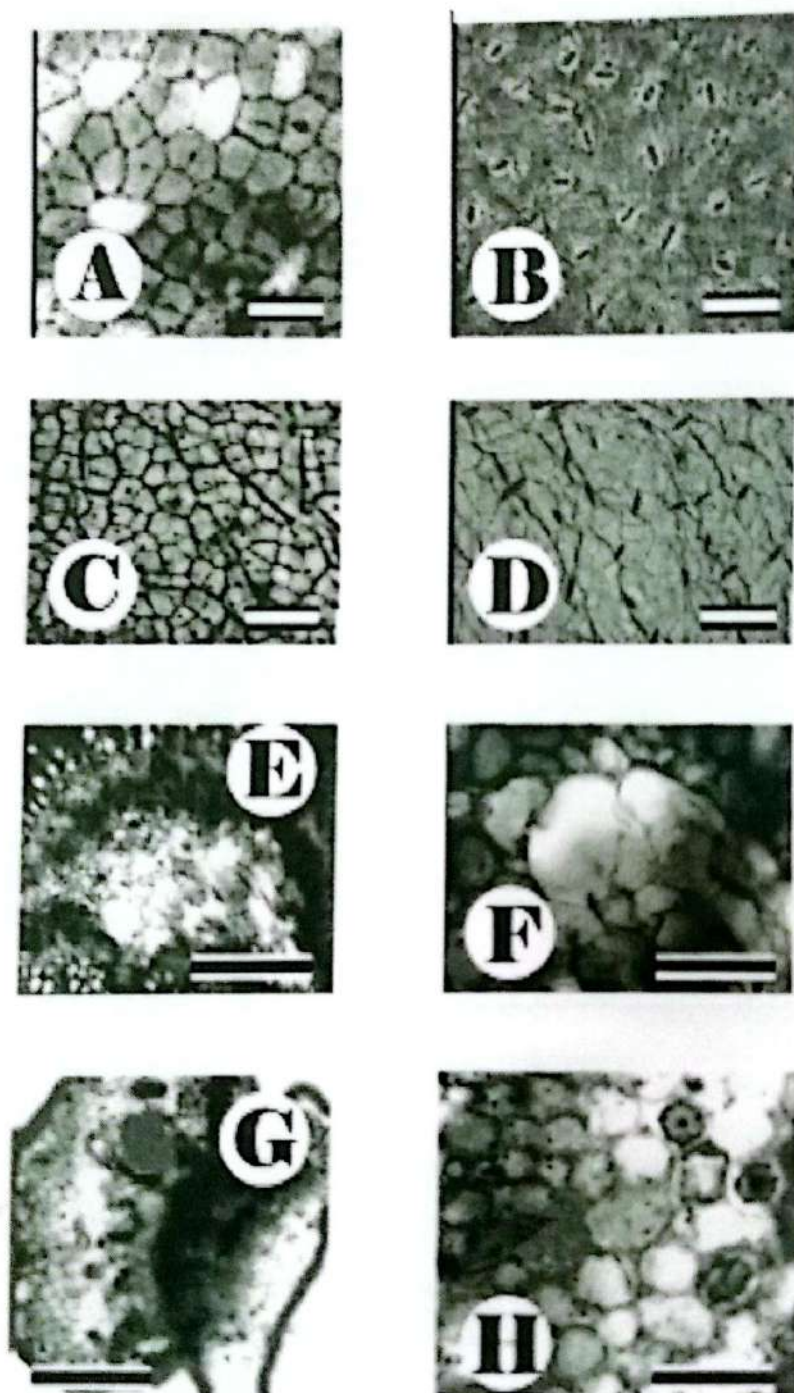


Fig. 1: Leaf epidermis and midrib anatomical characteristics of *Lagerstroemia*. A, B, G, H: *L. indica*, C, D, E, F: *L. speciosa*. A, B and C: showed striations in the leaf epidermis; F: reveals the radiant globular parenchyma cells in *L. speciosa*. H: shows crystal deposits within parenchyma cells of *L. indica*. A, C (adaxial surface of leaf epidermis), B, D (abaxial surface of leaf epidermis), E-H (leaf midrib). Scale bars: A-D=20µm. Others=75µm.

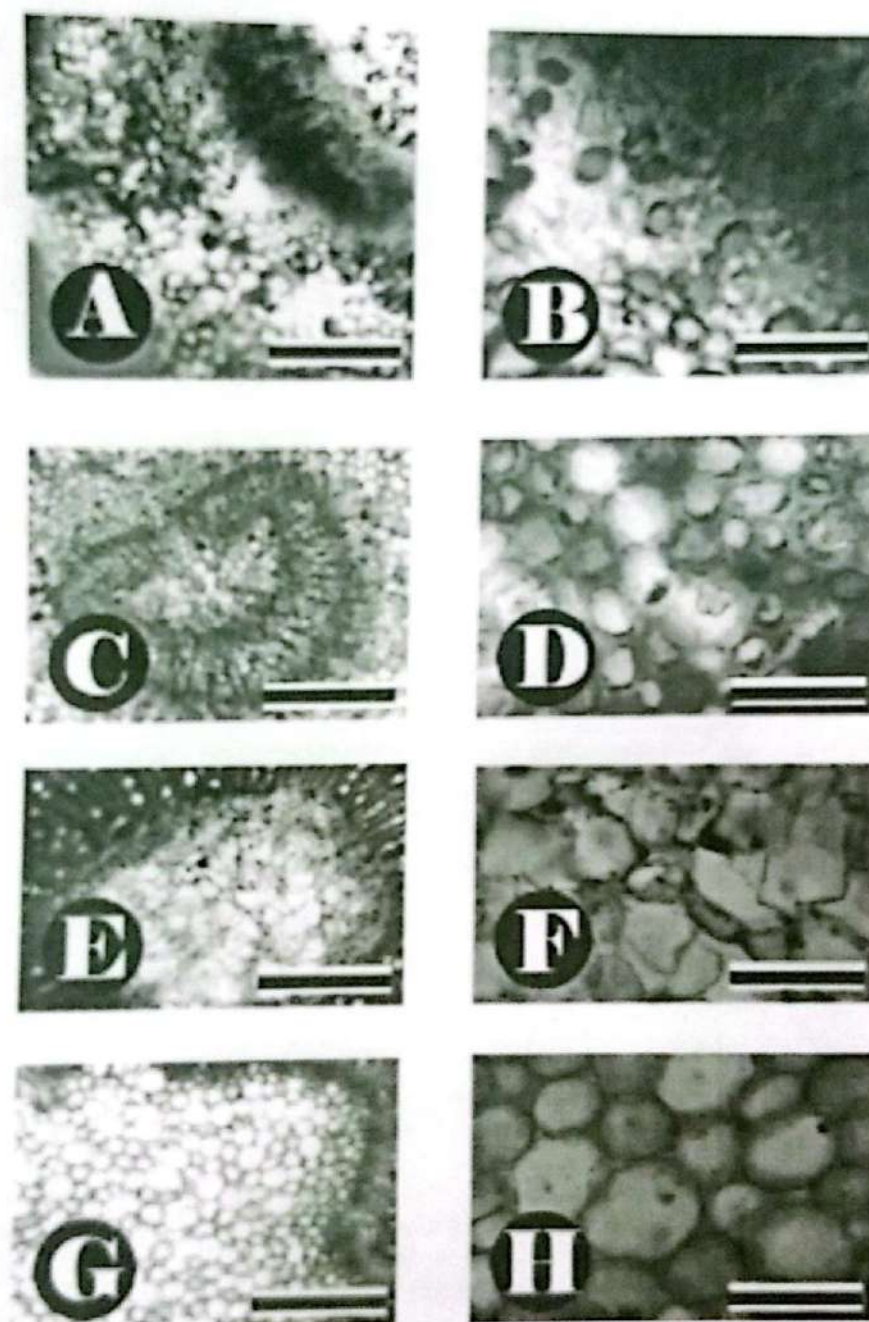


Fig. 2: Some anatomical features of the petiole and stem of *Lagerstroemia*. A, B, E, F: *L. indica*, C, D, G, H: *L. speciosa*. A-D: showed scattered parenchyma cells in the petiole of *L. indica* and *L. speciosa*. G, H showed globular parenchyma cells in the stem of *L. speciosa*. Note the presence of cell inclusions within the parenchyma of stem. Scale bar: 75μm.

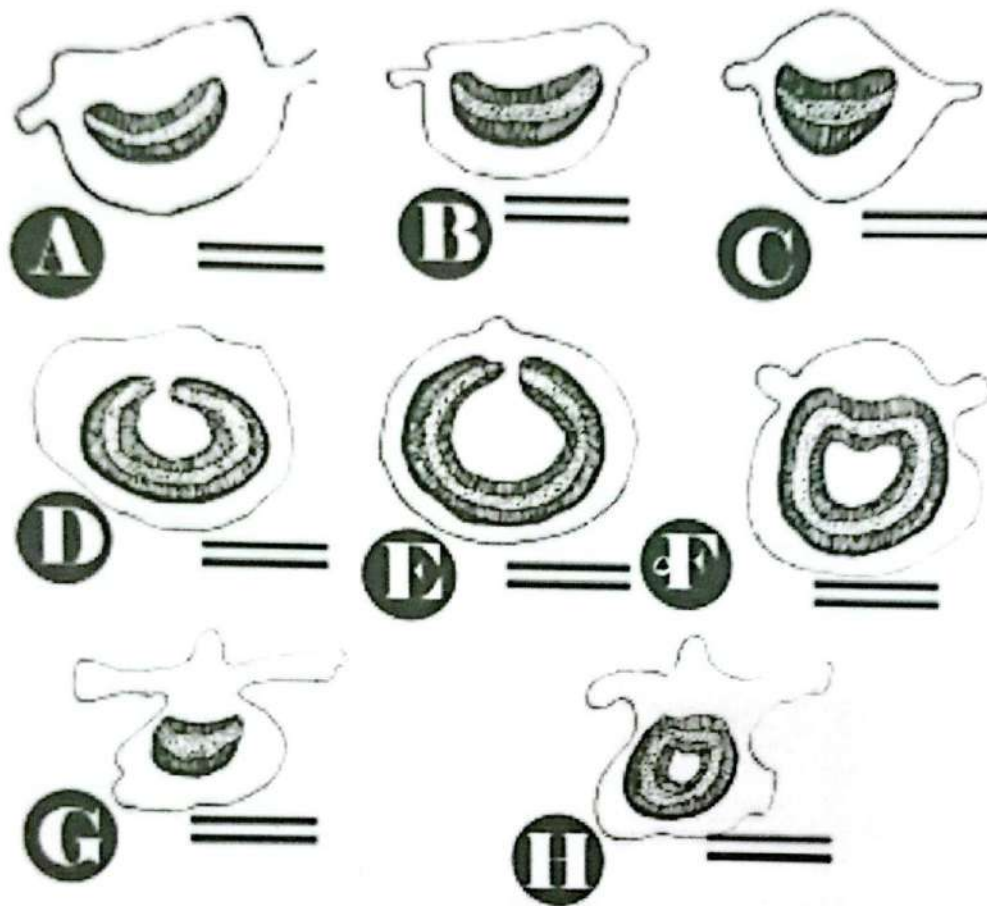


Fig. 3: Line Diagrams of petiole and midrib of *Lagerstroemia*. A-F: petioles; A, D: proximal end, B, E: median position and C, F: distal end. G, H: midribs. A-C and G: *L. indica*. D-F and H: *L. speciosa*. The vascular bundle is bicollateral in the two species. Horizontal lines represent phloem and dotted lines stand for xylem. Solid line subtending the vascular bundle is sclerenchymatous ring. Scale bar: 50μm.