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Observations on the foliar architecture and micro-morphology of *Diospyros lanceifolia* Roxburgh (Ebenaceae)

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Abstract

Foliar architecture and micro-morphological characteristics *of Diospyros lanceifolia* Roxburgh (Ebenaceae) were studied. Qualitative and quantitative characters of veins, vein order, areolation, epidermal cell and cell wall nature, length, breadth and number of epidermal cell; stomatal distribution, type, stomatal frequency and stomatal index were taken into consideration. The observed characters were found useful in distinguishing the species in the genus *Diospyros* Linnaeus of Ebenaceae.

***Key words*:** Foliar architecture, Micro-morphology, *Diospyros lanceifolia*, Ebenaceae, Assam.

INTRODUCTION

Commonly known as "Persimmon" or "Ebony", the genus *Diospyros* Linnaeus (Ebenaceae) comprises of 768 species distributed throughout the tropical forests in Asia, Australia, Pacific Islands and Africa including Madagascar and the Indo-Pacific region (Duangjai *et al.* 2006). Fruits of *Diospyros* are the most nourishing parts and included in diet of many inhabitants of in the tropical region. Various species of *Diospyros* are used in folk medicines and constitute a part in formulation of drugs in Ayurvedic system of medicines **(**Chopra *et al.* 1956; Mallavadhani *et al.* 1998; Bhushan *et al.* 2005; Laloo *et al.* 2006)**.** The uniqueness of the genus is the elaboration of a large number of pentacyclic triterpenes and juglone based 1, 4-naphthoquinone metabolites (Ravikumar *et al.* 2014).

Diospyros lanceifolia Roxburgh is a medicinally important species of Ebenaceae. This lowland rainforest species is distributed throughout the tropical region of the world from India and Sri Lanka in the northwest through SE Asia (Myanmar, Indo-China, Thailand, Peninsular Malaysia) to Sumatra, the Lesser Sunda Islands and Sulawesi (Gratiana *et al.* 2004). It is a medium evergreen tree (48 – 50 m height) with hard and blackish wood; young shoots pubescent; branchlets and bark with peculiar smell. Bark blackish, rough with more or less parallel vertical fissures 0.8 cm – 1.3 cm apart. Leaves simple lanceolate, ±13 x 5 cm, entire, acute, base rounded, glabrous, shining above; midrib depressed above, conspicuous beneath, lateral nerves slender; petiole ± 0.6 cm long. Plants dioecious with inferior unisexual flowers. Pedicels articulated under flower. Male inflorescence ±0.5 cm long with 3 – 10 crowded flowers; calyx divided into 4 valvate triangular lobes; corolla salver-shaped, short and twisted to right; stamens 5, unequal, commonly inserted at the base or higher up in

corolla tube, basifixed, rarely short, ovary rudimentary. Female inflorescence ±0.6 cm long with 3 – 7 flowers; calyx divided into 4 valvate triangular lobes; ovary, glabrous; style 1, usually short, distally simple or slightly bifid; stigma bilobed. Berries subglobose to ovoid, slightly accrescent, smooth, shiny, astringent and fleshy.

Taxonomic importance of foliar epidermal characters in angiosperms has been emphasized by many authors including Stace (1961), Shah & Kothari (1975), Lavania (1990), Padmini & Rao (1995) and Paul & Devi (2013). Its role in differentiation of taxa upto the rank of species is well established (Adedeji 2004; Kadiri 2006). Stomata and trichome types have been widely emphasized as important taxonomic attributes by various workers (Shah & Kothari 1975; Lavania 1990; Padmini & Rao 1995; Garg 2010; Paul & Devi 2013). Not much has been elicited on the foliar architecture and epidermal characters of *Diospyros lanceifolia* and hence the present study is an attempt to evaluate foliar architecture and epidermal features as an aid to identification and delimit the species in the genus *Diospyros* Linnaeus of Ebenaceae.

MATERIALS AND METHODS

Flowering twigs of *D. lanceifolia* were collected from various localities of Kamrup district of Assam. Voucher specimens were processed following standard herbarium techniques (Jain & Rao 1977) and were identified with the help of relevant literatures (Hiern 1872; Francis 2002) and previously identified specimens at GUBH, ASSAM, CAL and also with images of herbarium specimens of online databases of various herbaria like K, JSTOR and EOL.

To study the foliar architecture fresh and mature leaves were cleared using techniques suggested by Bersiar & Bocquet (1960) with slight modification. Small pieces of leaves (about 3 cm) were taken from the mid-rib region at the apex, middle and basal portion and were treated with 5 % aqueous NaOH for overnight in an oven at 32º C and were later soaked in aqueous solution of HNO_3 in various concentrations (30 %, 50 %, 70 %, 90 % and 100 %) and boiled. After repeated washing in distilled water the materials were dehydrated through the ethanol grade and kept there until the disappearance of chlorophyll. Permanent slides were prepared from the treated materials and microphotographs were taken. Venation pattern was described using terminology as suggested by Hickey (1973), Dilcher (1974) and Melville (1976).

To study foliar epidermal characters fresh and mature leaves were cleared using techniques suggested by (Boulos & Beakbane 1971) with slight modification. For this both upper and lower epidermal peelings were made either mechanically or by scrapping with the help of a blade using 10 % aqueous HNO_3 solution. The peels were then stained with 1 % aqueous saffranine solution and were mounted with 1% glycerin after proper washing. Microphotographs were taken from the prepared slides at different magnifications under the microscope. The nature and distribution of stomata, epidermal cells, guard cells, subsidiary cells, cell size, and stomatal index were worked out for both upper and lower epidermises. For description of stomata terminologies suggested by Hickey (1973), Stace (1984) and Prabhakar (2004) were followed.

Stomatal Frequency: The stomatal frequency was calculated by following the formula of Ghosh & Davis (1973).

$$\textbf{Stomatal Frequency (S.F.)} = S/A$$

Where, S = Number of stomata per field
A = Area of the field

Stomatal Index: Stomatal index was calculated by following the formula of Dilcher (1974) and Salisbury (1927) with little modification**.**

$$\textbf{Stomatal Index (S.I.)} = \frac{S}{E+S} \times 100$$

Where, S = Number of stomata per unit area

E= Number of epidermal cells per unit area

Guard Cell Area: The guard cell area were calculated by following the formula of Franco (1939).

$$\textbf{Guard Cell Area (GCA)} = L \times B \times K$$

Where, L = Length of the guard cell

B = Breadth of the guard cell

K = Franco's Constant (K= 0.78524)

RESULTS AND DISCUSSION

Foliar Architecture:

The leaves of investigated species were unicoastate reticulate and the major venation pattern was pinnate-brochiodromous (Plate 1). The primary vein (1º) was stout and straight whereas 10 – 12 secondary veins (2º) were arranged alternately on either sides of primary veins forming smooth arch. The secondary veins (2º) were of reticulodromous type with basally decreasing irregular spacing. Weak intersecondaries were also observed. The tertiary veins (3º) were alternate percurrent type with sinuous vein course, forming obtuse angle to primary (1º) which were inconsistent; quaternaries (4º) regular polygonal reticulate type, arise at right angles to form orthogonal reticulations. Quineries (5º) and senaries (6º) were of thin dichotomizing type. On the dorsal side of the leaf, secondary, tertiary and quaternary arches form loops. The areoles were forming a contiguous field over most of the area of leaf which are 5 or more sided. The freely ending ultimate veins of the leaves were the senaries (6º) which are either linear (unbranched) or rarely once branched.

Foliar Micro-morphological Characteristics of Epidermis:

Qualitative and quantitative micro-morphological characteristics of epidermis were presented in tabular forms [Tables 1 & 2].

Epidermis was single layered with cells of both the surfaces were mostly penta- and octagonal. Cell walls were prominent and straight. There was no definite pattern of arrangement of epidermal cells. The length of epidermal cell ranges from 30.72 µm to 59.53 µm on lower surface whereas on upper surface it ranges from 31.71 µm to 60.80 µm. The L/B ratio of epidermal cells on upper surface and lower surface was ± 0.51.

Stomata:

Leaves hypostomatic, stomata were uniformly distributed in intercoastal areas but occasionally a few stomata may also present in the coastal areas. The type of stomata was anomocytic, and they were widely separated from each other by epidermal cells. The size of stomata was 90.16-91.45 µm× 59.78- 69.58 µm. The size of stomatal aperture on the lower surface

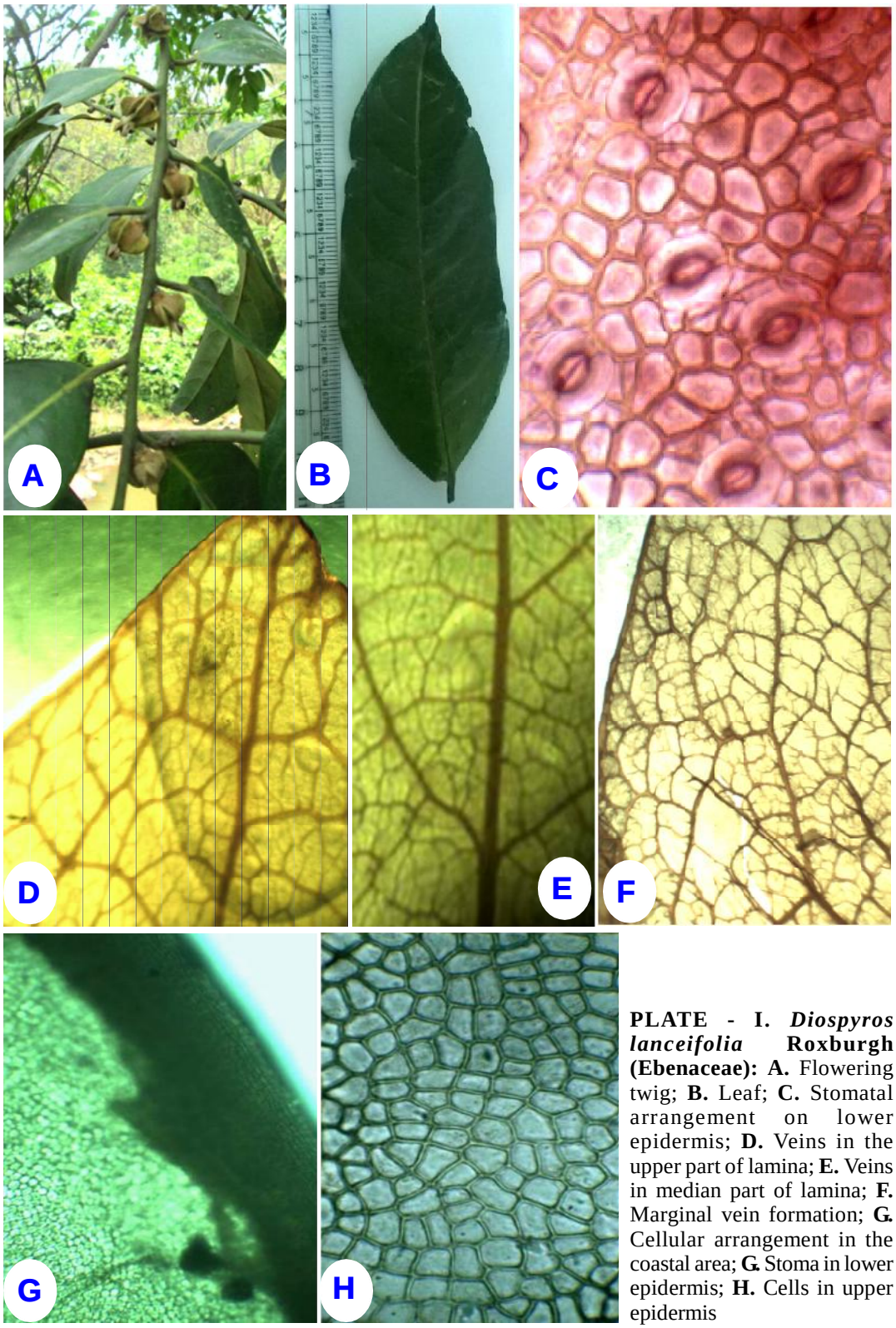

PLATE - I. *Diospyros lanceifolia* Roxburgh (Ebenaceae): A. Flowering twig; **B.** Leaf; **C.** Stomatal arrangement on lower epidermis; **D.** Veins in the upper part of lamina; **E.** Veins in median part of lamina; **F.** Marginal vein formation; **G.** Cellular arrangement in the coastal area; **G.** Stoma in lower epidermis; **H.** Cells in upper epidermis

Table 1. Qualitative foliar micro-morphological data of *D. lanceifolia* Roxburgh

Surface	Epidermal cells		Coastal Area	Tricho-me	Stomatal Size	Guard Cell	Type of stomata
Upper	Polygonal	Prominent & Straight	Distinct	-	-	-	-
Lower	Polygonal	Straight		-	Unequal	Unequal	Anomocytic

Table 2. Quantitative values for foliar micro-morphology of *D. lanceifolia* Roxburgh [*Abbreviations used*: ST= stomata; EC= epidermal cell; SI= stomatal index; SF= stomatal frequency; SS= stomatal size; SA= stomatal aperture; GCA= guard cell area]

Surface	ST/ mm^2	EC/ mm^2	SI/ mm^2	SF/ mm^2	SS(µm)		EC (µm)		SA(µm)		L/B ratio			GCA
					L	B	L	B	L	B	ST	EC	SA	
U	-	1823	-	-	-	-	31.71 ± 0.62	60.80 ±1.22	-	-	-	0.52	-	-
L	291	1853	306.70	96.36	91.45 ± 0.83	69.58 ± 0.46	30.72 ± 0.62	59.53 ± 0.95	0.78 ± 0.02	0.44 ± 0.01	1.31	0.52	1.77	0.25

was 0.78 µm × 0.44 µm. The L/B ratio of stomata and stomatal aperture were 1.31 and 1.77 respectively. The stomatal frequency ranged from 79.54 to 96.36 per sq mm whereas the stomatal index ranged from 256.42 to 306.70 per sq mm.

It has been observed that trichomes were totally absent on both the surfaces of lamina and with anomocytic stomata. Shape of epidermal cell was irregular on both the surfaces with straight and distinct cell walls. Qualitative as well as quantitative characters like cell size, cell number, stomatal number and stomatal size etc. can be used in distinguishing the species from other species of the genus *Diospyros* Linnaeus of Ebenaceae.

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