Foliar micromorphological studies in some species of *Premna* Linnaeus (Lamiaceae) and their taxonomic implications

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Abstract

Foliar micromorphological study of six species of the genus *Premna* L. viz., *P. cordifolia* Roxb., *P. divaricata* Wall. ex Schauer, *P. mollissima* Roth, *P. pyramidata* Wall. ex Schauer, *P. racemosa* Wall. ex Schauer, and *P. resinosa* Schauer was carried out and illustrated for the first time using scanning electron microscopy with a view to explore and evaluate the taxonomic significance of foliar epidermal features in the genus. Leaves of all the six species are hypostomatic. Two types of stomata, paracytic and anomocytic are generally concentrated on abaxial surfaces. Two basic types of trichomes are identified: glandular and non-glandular trichomes. The non-glandular trichomes can be further subdivided into two subtypes: simple unbranched attenuate and stellate branched trichomes. Two subtypes, capitate and peltate glandular trichomes are recognized. The comparative study shows the epidermal features possess taxonomic significance and could be used as an important supportive taxonomic character to delimit the species of *Premna* L. An artificial bracketed key for distinguishing the taxa is provided.

Key words: Foliar micromorphology, *Premna*, Scanning electron microscopy (SEM), systematic.

INTRODUCTION

The foliar epidermis offers one of the most significant taxonomic characters from the biosystematic point of view. Features of epidermal cells and stoma are widely used as taxonomic evidences and the taxonomic studies of a number of families have been made on the basis of leaf epidermis (Baranova 1972; Metcalfe & Chalk 1950; Stace 1984; Hatamneia et al. 2008; Judd et al. 2008; Moon et al. 2009; Shaheen et al. 2009; Thakur & Patil 2011). The systematic and phylogenetic significance of the indumentum is well known in Lamiaceae s.l. and closely related families (Metcalfe & Chalk 1950; El-Gazzar & Watson 1970; Ahmad 1974, 1978; Mathew & Shah 1981, 1983; Abu-Asab & Cantino 1987; Cantino 1990; Rahn 1992; Gairola et al. 2009; Moon et al. 2009; Xiang et al. 2010; Osman 2012).

Trichomes are very common and widely distributed on the aerial parts of the members of Lamiaceae s.l. and are generally distinguished as glandular and non-glandular (Mathew & Shah 1981; Cantino 1990; Navarro & El Ouali 2000; Xiang et al. 2010). Non-glandular trichomes are more common than glandular trichomes in Lamiaceae s.l. (Mathew & Shah 1981; Xiang et al. 2010).
According to Metcalfe & Chalk (1950) the diacytic stomata are most common in Lamiaceae and often intermixed with anomocytic. In 1970, El-Gazzar & Watson added anisocytic type stomata to Lamiaceae. Subsequently, Inamdar & Bhat (1972) reported diacytic, transitional between paracytic and diacytic, and anomocytic stomata in Lamiaceae. Moon et al. (2009) reported five types of stomata viz. actinocytic, anomocytic, anisocytic, diacytic, and dialeelocytic, in Lamiaceae, of which actinocytic, and dialeelocytic are addition to the previous report.

The genus *Premna* Linnaeus belongs to Lamiaceae s.l. with ca. 200 species worldwide and is distributed mainly in tropical and subtropical Asia, Africa, Australia and the Pacific Islands (Harley et al. 2004). In India, it is represented by 31 species and 6 varieties (Rajendran & Daniel 2002). The genus was first established by Linnaeus (1771) based on *P. serratifolia* Linnaeus.

Detailed study on comparative micromorphology of foliar epidermis and their systematic relevance within the same genus are however very scarce. In 1981, Mathew & Shah for the first time described the structure of trichomes on different organs in six species of *Premna*. Later, Yashodhara et al. (2004) studied structure and organographic distribution of trichomes on different vegetative and floral parts in 26 taxa belong to seven genera including ten taxa from the genus *Premna* of the tribe Viticeae, but only few aspects on foliar micromorphology were discussed.

There has been no comprehensive foliar epidermal study in the genus *Premna*. Therefore, the intent here is to present characterization and illustrations of the foliar micromorphology of some selected species of *Premna* using scanning electron microscopy (SEM); to determine the extent and pattern of these features and to describe the variation in these features and their systematic implications.

**MATERIALS AND METHODS**

Dried herbarium specimens were obtained from the Herbarium, Royal Botanic Gardens Kew (K), listed in Table 1. The identities of the specimens were authenticated at K with the assistance of Dr. Alan Paton. The leaf samples (ca. 8 mm²) were taken from the mid-lamina position, which is considered to be the least variable (Wilkinson 1979), then boiled in water for rehydration. All samples were dehydrated through a graded ethanol series. Two sections of both adaxial and abaxial surfaces were prepared for each specimen. The sections were mounted on electron microscope stubs using double sided adhesive tabs. The stubs were then coated with approximately 350Å gold in a Polaron SC500 sputter coater and the samples were examined under Scanning Electron Microscope (model Hitachi S4300) and photographed at Center for Microscopy and Analysis in the Trinity College Dublin (TCD), University of Dublin, Ireland.

**Table 1.** List of species investigated along with their sources

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Voucher specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Premna cordifolia</em></td>
<td>Darjeeling, India</td>
<td>Clarke 40762</td>
</tr>
<tr>
<td><em>P. divaricata</em></td>
<td>Andaman, India</td>
<td>Parkinson 662</td>
</tr>
<tr>
<td><em>P. mollissima</em></td>
<td>Assam, East Bengal, India</td>
<td>Unknown collector, <em>s. n.</em></td>
</tr>
<tr>
<td><em>P. pyramidata</em></td>
<td>Maymyo Plateau, Myanmar</td>
<td>Lace 6246</td>
</tr>
<tr>
<td><em>P. racemosa</em></td>
<td>Sikkim, India</td>
<td>Unknown collector, <em>s. n.</em></td>
</tr>
<tr>
<td><em>P. resinosa</em></td>
<td>Kilifi Malindi, Kenya</td>
<td>Robertson 5913</td>
</tr>
</tbody>
</table>

Figure 2

Foliar micromorphology of *Premna*
RESULTS

The leaf micromorphology of six species of the genus *Premna* has been studied. The descriptions emphasize the mature state of the adaxial and abaxial leaf surfaces. The images of the foliar surfaces of *Premna* are presented in Figures 1 – 3 below, and the observations of characteristics are summarized in Table 2.

The general classification scheme of trichomes is based on El-Gazzar & Watson (1970); and Pyne (1978); but the typological classification of glandular trichomes is based on Cantino (1990).

*Premna cordifolia* Roxburgh  
Leaves: hypostomatic  
[Figures 1A – D]

**Adaxial**: glabrous; cuticle smooth, tessellated, shallow grooves indicating undulated anticlinal walls; epicuticular wax minutely present.

**Abaxial**: glabrous; cuticle smooth, tessellated, cell walls distinctly sinuate. **Stomata**: paracytic; frequent, large, somewhat sunken, outline broadly elliptic or rarely oblong, rim distinctly prominent, aperture long and broad.

*Premna divaricata* Wallich ex Schauer  
Leaves: hypostomatic  
[Figures 1E – H]

**Adaxial**: glabrous; a few attenuate trichomes rarely scattered, mainly along the veins, base broad; cuticle distinctly reticulate-ridged, cells polygonal with straight anticlinal cell walls, lumina inconspicuously coarse.

**Abaxial**: glabrous; cuticle conspicuously ridged, cells irregular, anticlinal cell walls curved. **Stomata**: paracytic; frequent, mostly large, a few small, outline broadly elliptic, rim distinctly prominent, aperture narrow, rarely broad.

*Premna mollissima* Roth  
(P. latifolia* Roxburgh)  
Leaves: hypostomatic  
[Figures 2A – D]

**Adaxial**: glabrous; along the veins a few attenuate, bicellular, eglandular trichomes with warty surface rarely scattered; cuticle tessellated with shallow grooves, conspicuously ridged along the veins, cells polygonal, anticlinal cell walls straight to somewhat curved.

**Abaxial**: glabrescent; small, sessile, capitate gland with 4-celled head rarely present; a few attenuate, multicellular, somewhat curved eglandular trichomes scattered mainly along the veins; cuticle tessellated, cells irregular with undulated cell walls. **Stomata**: anomocytic, rarely paracytic; frequent, large, outline broadly elliptic, rim prominent, aperture narrow.

*Premna pyramidata* Wallich ex Schauer  
Leaves: hypostomatic  
[Figures 2E – H]

**Adaxial**: glabrous; a few sessile, capitate glandular trichomes with 2 - 3-celled head; and small, sub-sessile, with short arms, stellate trichomes rarely scattered; cuticle smooth, cells polygonal, anticlinal cell walls straight.

**Abaxial**: densely stellate; large, sub-sessile, profusely branched, long armed, stellate trichomes with long central ray, frequently distributed; cuticle ridged, much pronounced along the veins.

Table 2. Summary of the micromorphological features of investigated species of *Premna* L. (Lamiaceae). [Abbreviations used: **AB** = abaxial; **AD** = adaxial; **AT** = attenuate; **ST** = stellate; **CP** = capitate; **PL** = peltate; **RD** = ridged; **RR** = reticulate-ridged; **SM** = smooth; **ST** = striated; **TS** = tessellate]

<table>
<thead>
<tr>
<th>Species</th>
<th>E glandular trichomes</th>
<th>Glandular trichomes</th>
<th>Cuticle</th>
<th>Stomata type</th>
<th>Stomatal density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type &amp; Occurrence</td>
<td>No. of cells</td>
<td>Frequency (abaxially)</td>
<td>Type &amp; Occurrence</td>
<td>Peltate head (No. of cells)</td>
</tr>
<tr>
<td><em>P. codifolia</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. divaricata</em></td>
<td>AT (AD)</td>
<td>Rare</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. mellissima</em></td>
<td>AT (AD + AB)</td>
<td>Unicellular</td>
<td>Scattered</td>
<td>CP (AB)</td>
<td>4</td>
</tr>
<tr>
<td><em>P. pyramidata</em></td>
<td>ST (AD + AB)</td>
<td>Multicellular</td>
<td>Frequent</td>
<td>CP (AD)</td>
<td>2-3</td>
</tr>
<tr>
<td><em>P. racemosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CP (AB)</td>
<td>2</td>
</tr>
<tr>
<td><em>P. resinosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PL (AD + AB)</td>
<td>13-14</td>
</tr>
</tbody>
</table>
cells irregular with undulated anticlinal cell walls. Stomata: anomocytic, rarely paracytic; frequent, large, outline broadly elliptic, somewhat sunken, rim prominent, aperture narrow.

**Premna racemosa** Wallich ex Schauer Leaves: hypostomatic [Figures 3A – D]

**Adaxial:** glabrous; cuticle distinctively reticulate-ridged; cells polygonal with straight anticlinal cell walls, lumina smooth.

**Abaxial:** glabrous; sessile, capitate glandular trichomes rarely scattered, head 2-celled; cuticle conspicuously striated, cells polygonal, anticlinal cell walls straight; epicuticular wax particles present. Stomata: paracytic; abundant, large, outline elliptic, rim distinctly prominent, aperture narrow.

**Premna resinosa** (Hochstetter) Schauer Leaves: hypostomatic [Figures 3E - H]

**Adaxial:** glabrous; sessile, multicellular, dome-shaped peltate glandular trichomes rarely scattered, base sunken; cuticle tessellated, cells irregularly shaped, anticlinal cell walls sunuate.

**Abaxial:** mostly glabrous; sessile, multicellular, saucer-shaped, peltate glandular trichomes frequently distributed, almost sunken; cuticle tessellated, cells irregularly shaped, anticlinal cell walls sunuate. Stomata: paracytic; abundant, large, outline broadly elliptic or oblong, rim distinctly prominent, aperture narrow.

**DISCUSSION**


The present investigation on six species of the genus *Premna* L. is quite significant. The micromorphological characters of each of the six species are unique and thus, can be used as important supportive taxonomic characters for the delimitation of each of these species.

The epidermal cells mostly polygonal adaxially and irregular on abaxial surfaces. Anticlinal cell walls are either straight or undulate and curved. Cuticle patterns are tessellated on both surfaces in *P. cordifolia, P. mollissima,* and *P. resinosa* while adaxially smooth and ridged on abaxial surfaces in *P. pyramidata;* reticulate-ridged on adaxially in *P. divaricata* and *P. racemosa* but abaxially ridged in earlier and stariated in latter species (Table 2; Figures 1-3).

The stomata of the Lamiaceae are described by Metcalfe & Chalk (1950) as mostly diacytic, and often intermixed with anomocytic. Cantino (1990) concluded that anomocytic and diacytic were the most frequent types in both Lamiaceae and Verbenaceae. The present investigation reveals that the leaves are hypostomatic in all *Premna* species examined, and stomata are mostly paracytic except *P. mollissima* and
Figure 3

Foliar micromorphology of *Premna*
P. pyramidata which are predominant with anomocytic type (Table 2; Figures 1-3); which incongruence with the earlier report. 

Trichomes are one of the most important features which contribute to passive resistance of plants to pathogens, pests and drought (Levin 1973). In most of the species trichomes are much denser on the abaxial surfaces, which tend to diminish the importance of trichomes as a sun-shade adaptation (Husain et al 1990). Many plant groups show great diversity in their indumenta, some of which are of taxonomic significance. While ecological variations may affect the degree of hairiness, the type of hair is usually constant in species group (Stace 1965; Okpon 1969) and the presence or absence and types of trichomes on the epidermal surfaces as taxonomic tools (Adeleke et al. 2007; Saheed and Iloho 2010). Metcalfe & Chalk (1950) has long suggested that the types of epidermal trichomes can frequently delimit different taxonomic levels in plant. Trichome micromorphology has been employed by Mathew & Shah (1981) to delimit species in the genus Premna; Xiang et al. (2010) to delimit species of the Asian genus Chelonopsis; and different taxa in the tribe Viticeae by Yashodhara et al. (2004).

In the present study, presence or absence of trichomes, as well as their types can be useful in characterizing the species. In the genus Premna, glandular and eglandular trichomes are frequently encountered only on the abaxial surfaces of P. pyramidata (Figures 2G-H) and P. resinosa (Figures 3G-H) respectively, while rest of the species are almost lack of trichomes on both surfaces.

The present study reveals (Table 2) two main trichome types - glandular and eglandular and these can be further subdivided as follows.

I. Glandular trichomes

The glandular trichomes constitute an important taxonomic character in the family Lamiaceae and Verbenaceae (Cantino 1990; Navarro & Oualidi 2000; Xiang et al. 2010). In the present investigation two subtypes of glandular trichomes are recognised:

a) Capitate: A sessile or sub-sessile glandular trichome with a spherical head. In P. mollissima (Figure 2C) and P. racemosa (Figures 3C-D) they have been found very rarely on the abaxial surfaces only and in P. pyramidata adaxially only (Figure 2F). The term “Capitate” follows Inamdar (1969) and Pyne (1978).

b) Peltate: Scales or lepides; sessile or stalked trichomes with flattened heads which consist either of a single cell developed as a flat structure, or of a varying number of cells arranged in one or more layers with circular- or quadrangular- or irregular wavy-margins. In Premna this type of trichomes are only observed in P. resinosa (Figures 3E-H) where they occur on both surfaces, adaxially the trichomes are dome-shaped (Figures 3F), but abaxially they are saucer-shaped and almost sunken (Figures 3G-H). The term “Peltate” follows Pyne (1978). Following the descriptions and typology of Abu-Asab & Cantino (1987); and Cantino (1990), and based on the cell numbers and the cell-wall configuration, the peltate glands correspond to types 2, 3, 4, 8 and 11. The present investigation encounters peltate glands with more than 11 cells in P. resinosa (Figure 3E-H), which clearly differs with the previous observations.

II. Eglanular trichomes

The following two subtypes of eglandular trichomes observed in this investigation.

a. Attenuate: Simple, unbranched trichomes, gradually tapered from a conspicuous swollen base; short to long and straight to curved. Straight attenuate trichomes were observed adaxially in *P. divaricata* (Figures 1E-F), and somewhat curved attenuate trichomes were found on both surfaces in *P. mollissima* (Figures 2A-D). The term used here follows Pyne (1978).

a) Stellate: Trichomes which are star-shaped or branched as a star with rays projecting from center. Stellate bunched trichoes are observed in *P. pyramidata* on both surfaces, while short armed trichomes rarely observed adaxilly (Figure 2E) and profusely branched, long armed stellate trichmes are densely distributed on the abaxial surface (Figures 2G-H). The terminology “Stellate” used here follows El-Gazzar & Watson (1970).

The distinct type “stellate” trichomess are observed only in *P. pyramidata*, by which it can be easily distinguished from other species. Similarly *P. resinosa* is also distinctive from other species having dome-shaped or saucer-shaped 13-14-celled peltate glandular trichomes.

All *Premna* species examined are almost glabrous on both surfaces except *P. pyramidata* in which abaxial surface is densely stellate.

Based on the most reliable features that are reported in the present study, an artificial bracketed key is thus presented below to delimit the species investigated in the genus *Premna* Linnaeus.

1. Leaves pubescent with stellate eglandular trichomes  ..........  *P. pyramidata*

1. Leaves almost glabrous, lacking eglandular trichomes  ..........  2

2. Stomata mostly anomocytic, rarely paracytic  ..................  *P. mollissima*

2. Stomata paracytic  ..............................................  3

3. Cuticular pattern tessellated on both surfaces  ..................  4

3. Cuticular pattern not tessellated  ................................  5

4. Peltate glandular trichomes present on both surfaces  ..........  *P. resinosa*

4. Leaf surfaces glabrous  ........................................  *P. cordifolia*

5. Abaxial surface conspicuously striated, anticlinal cell walls straight  ...........  *P. racemosa*

5. Abaxial surface ridged, anticlinal cell walls curved  ............  *P. divaricata*

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LITERATURE CITED


