RUBIACEAE

The family Rubiaceae comprises 500 genera and 6000 species (Willis, 1966) distributed throughout the world. The family is economically important for timber trees such as Adina cordifolia and *Mitragyna parviflora*. Of these 6000 species, Tognini (1897) investigated 1 species, Pant and Mehra (1965) 14 species and Bir Bahadur et al 26 species for stomatal structure and development (see following table for details). The present study deals with the structure and ontogeny of stomata in 14 genera and 33 species. The study is made largely on leaves but in some case hypocotyl and cotyledons have also been studied (See list under Materials).

Mean values of 15 observations (lower epidermis only) of the species studied showing percentage of different types of stomata, stomatal frequency and index per mm², size of guard and epidermal cells in *4*, nature of epidermal walls and surface are charted in table to explanation of text figures 1:1 to 1:3. Past and present work in the Rubiaceae has been summarised as under:

<table>
<thead>
<tr>
<th>Species of the Rubiaceae studied - past and present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Past:</strong></td>
</tr>
<tr>
<td>I. Tognini (1897) * Coffea arabica</td>
</tr>
<tr>
<td>II. Pant &amp; Mehra (1965) ° 1. Anthocephalus cadamba</td>
</tr>
</tbody>
</table>
2. *Borreria hispida*
3. *Catesbaea spinosa*
4. *Cinchona succiribra*
*5. *Coffea arabica*
6. *C. bengalensis*
7. *Gardenia gumifera*
8. *Hamelia patens*
9. *Ixora coccinea*
°10. *Mussaenda frondosa*
°11. *Oldenlandia corymbosa*
12. *Paederia foetida*
13. *Randia malabarica*
°14. *Rubia cordifolia*

13 species more than Tognini (1879) are worked out.

III. Bir Bahadur, Rajgopal and Ramayya (1971)

+ 1. *Anotis foetida*
° 2. *Borreria hispida*
+ 3. *Borreria stricta*
*4. *Coffea arabica*
+ 5. *Dentella repens*
6. *Diodia teres*
° 7. *Hamelia patens*
8. *Hedyotis nigricans*
° 9. *Ixora coccinea*
10. *Kohautia aspera*
°11. *Mussaenda frondosa*
+12. *Morinda tomentosa*
18 species more than Pant and Mehra (1965) are worked out.

**Present:**

IV.

+ 1. *Anotis foetida*
+ 2. *Anthocephallus indicus*
  3. *Asperula arvensis*
+ 4. *Borreria articularis*
+ 5. *B. stricta*
  6. *Cinchona officinale*
* 7. *Coffea arabica*
  8. *C. ejunoides*
  9. *Crucianella angustifolia*
+10. *Dentella repens*
  11. *Galium odoratum*
12. *G. palustre*
13. *Galium rubioides*
14. *G. tricornae*
15. *Gardenia resinifera*
°16. *Hamelia patens*
17. *Hymenodictyon excelsum*
°18. *Ixora coccinea*
19. *I. javanica*
20. *I. parviflora*
21. *I. stricta*
22. *Mitragnya parviflora*
23. *Morinda citrifolia*
+24. *Morinda tinctoria var. tomentosa*
°25. *Mussaenda frondosa*
°26. *Oldenlandia corymbosa*
27. *Pentas carneae*
+28. *P. lanceolata*
29. *P. lanceolata var.*
+30. *Rondeletia odorata*
°31. *Rubia cordifolia*
+32. *Sherardia arvensis*
33. *Xeromphis spinosa*

18 species more than the previous workers are worked out.

* Species worked out by all four workers 1
° Species worked out by II, III & IV 7
+ Species worked out by III & IV 7
OBSERVATIONS

I. EPIDERMIS:

(i) **Surface:** Leaves of *Asperula arevensis*, *Crucianella angustifolia*, *Dentella repens*, *Galium rubioides*, *G. tricornae*, *Gardenia resinifera*, *Morinda tinctoria* var. *tomentosa*, *Oldenlandia corymbosa*, *Pentas lanceolata*, *Sherardia arvensis*, *Borreria articularis* and *B. stricta* are amphistomatic. The leaves of the other species studied are hypostomatic.

(ii) **Structure:** The epidermal cells are polygonal and isodiametric or elongated in one direction. The elongated epidermal cells are arranged in longitudinal direction with their axes parallel to the organs. There is no definite pattern of arrangement of polygonal and isodiametric epidermal cells. The cuticle may be thin or thick and striated. The epidermal walls in surface view are either straight, arched or sinuous, thin or thick (Fig. 1:1 to 1:3). The epidermal cells contain chloroplasts.

(iii) **Dimensions:** The maximum length of the epidermal cells is 109 μ in *Anotis foetida* and minimum is 30.5 μ in *Galium rubioides*. The maximum breadth of epidermal cells is 76.3 μ in *Anotis foetida* and minimum is 13.9 μ in *Gardenia resinifera*. The epidermal cells of *Anotis foetida* have maximum length and breadth.
II. STOMATA:

1. Mature:

(i) **Distribution and orientation**: The mature stomata are uniformly distributed over the entire leaf surface in between the veins, sometimes over the finer veins but not over the main veins. The stomata are either widely separated from each other or lie close to each other forming distinct groups eg. in *Asperula arvensis* (Fig.1:1C), *Gardenia resinifera* (Fig.1:2A), *Mitragyna parviflora* (Fig.1:2K) and *Xeromphis spinosa* (Fig.1:3Q-R). The orientation of stomata is unidirectional in case of elongated epidermal cells and also in *Oldenlandia corymbosa* (Fig.1:2 Q-R), while in other cases the orientation of stomata is in any direction.

(ii) **Type**: The great majority of the stomata are paracytic but occasionally anomocytic and rarely anisocytic and diacytic types also occur (see Explanation to text figures 1:1 to 1:3). Stomata on the hypocotyl and cotyledons are anomocytic (Figs.1:1 L, R, T, U; 1:3H).

(iii) **Structure**: The paracytic stomata are flanked by 2-4 parallel subsidiary cells. The subsidiary cells are either non-contiguous at both ends (Figs.1:1C, W, X; 1:2I-M, Q; 1:3G, L) or contiguous at one (Figs.1:1D, D, H, K, O; 1:2B, G-I, L,O; 1:3I) or both (Figs.1:1A, C-D, E-F, H, J-Q, S, V-Z; 1:2A-R; 1:3A-P) poles. The paracytic stomata are either monocyclic (Figs.1:1A, C-D, E-F, H, J-Q, S, V-Z;
1:2A-B, D-M, O-R; 1:3A-C, E-K, M-P) or incompletely amphicyclic (Figs. 1:1J, K; 1:2B, E, K; 1:3I) or amphicyclic (Figs. 1:2C, N). The stomata become incompletely or completely amphicyclic due to the actual division of the subsidiary cells or rarely the adjacent epidermal cells assume the form of subsidiary cells as a result of readjustment during maturation of the epidermis. Anomocytic stomata are surrounded by 2-5 ordinary epidermal cells (Figs. 1:10, P, T, U; 1:2P). Anisocytic stomata are surrounded by 3 subsidiary cells of which one is distinctly smaller (Fig. 1:2P). Very rarely in Asperula arvensis, diacytic stomata occur (Pl. 1:1B). Subsidiary cells contain chloroplasts (Pl. 1:1B, D, E-H).

(iv) Abnormalities: Juxtapposed contiguous stomata (Figs. 1:1F; 1:30; Pl. 1:1C, D); single guard cells with or without pore (Figs. 1:2H, I; 1:30; Pl. 1:1G); division of the guard cell (Pl. 1:1F); arrested development and persistent stomatal initial (Pl. 1:1H) are noticed. Here, in the last case, the initial becomes persistent after cutting two subsidiary cells it develops chloroplast and uniform wall thickening and fails to divide to produce a pair of guard cells and hence it may be called persistent guard mother cell.

(v) Size of guard cells: The maximum length of guard cells of stomata is 53.8 μm in Hymenodictyon excelsum and Ixora javanica and the minimum is 20.7 μm in Gardenia resinifera. The maximum breadth of guard cells of stomata is 11.6 μm in
Borreria articularis and the minimum is 3.2 μm in Ixora javanica.

(vi) **Percentage, stomatal, frequency and index:** The percentage of paracytic stomata range from 82-100; of anomocytic 1-6; anisocytic 1-8 and diacytic 2. In the species of Borreria and Galium, the percentage of anisocytic stomata is below 1% and therefore not considered and mentioned in the table. The highest stomatal frequency is 624/mm\(^2\) in Gardenia resinifera and Mussaenda frondosa and lowest is 96/mm\(^2\) in Anotis foetida. The highest stomatal index is 50/mm\(^2\) in Pentas lanceolata var. and the lowest is 08/mm\(^2\) in Galium palustre.

2. **Development of stomata**

The protoderm cell divides unequally by a slightly curved wall on one side to produce a small lenticular initial and a large cell (Fig.1:2A, 0; Pl. 1:1A). The small lenticular cell contain dense cytoplasm and has a prominent nucleus, while the larger cell does not contain a dense cytoplasm and has no prominent nucleus (Pl.1:1A). The lenticular cell functions as a stomatal initial or meristemoid while the large cell forms an encircling subsidiary cell or differentiates into an ordinary epidermal cell.

(i) **Paracytic stoma:** The meristemoid enlarges and divides unequally by a curved wall to form a smaller and a larger cell (Figs.1:1V; 1:2A, 0; Pl. 1:1A). The larger cell
differentiates into a first subsidiary cell while the smaller enlarges and divides again by a curved wall either non intersecting at both the poles or intersecting at one or both the poles to produce two unequal cells (Figs. 1:1D, D₂; E-F, M, U, W, Y, Z; 1:2A, O; 1:3B, I). As a result of two divisions of the meristemoid, a group of three cells (triad) is produced, one middle cell and two flanking cells. The flanking cells differentiate as two subsidiary cells, while the central functions as a guard mother cell. The guard mother cell enlarges and divides by a straight wall parallel to the subsidiary cells to produce a pair of guard cells which then develop an intervening pore in between (Figs. 1:1Z; 1:3B, I). When the subsidiary cells are intersecting at both the poles, the resulting paracytic stoma becomes monocyclic. In a monocyclic paracytic stoma, when one of the subsidiary cells divide or an encircling cell which produced the meristemoid is present, the stoma becomes incompletely amphicyclic. The paracytic stoma becomes completely amphicyclic when both the subsidiary cells of a monocyclic paracytic stoma divide or one of them divides and the other is an encircling cell.

(ii) Diacytic stoma: The development of this type is similar to that of the paracytic one up to the triad stage. At this stage, the guard mother cell instead of dividing by a longitudinal wall parallel to the subsidiary cells divides by a transverse wall right angles to the two subsidiary cells to produce a pair of guard cells, thus resulting in a diacytic stoma (Pl. 1:1B).
(iii) **Anisocytic stoma**: The meristemoid after producing two subsidiary cells in a spiral fashion and contiguous at one of the poles does not function as a guard mother cell but produce a third small subsidiary cell in a spiral fashion on the third side. The central cell surrounded by three subsidiary cells, now, functions as a guard mother cell. The guard mother cell divides to form a pair of guard cells resulting in an anisocytic stoma (Fig. 1:2P; Pl. 1:1F).

(iv) **Anomocytic stoma**: The meristemoid directly functions as a guard mother cell without cutting off any subsidiary cells. If divides by a straight wall to form a pair of guard cells.

3. **Morphogenetic grouping**: The ontogeny of paracytic, diacytic and anisocytic stomata conforms to the mesogenous type as the subsidiary and guard cells are produced by the same meristemoid, while that of the anomocytic type is perigenous.

**DISCUSSION AND SUMMARY**

Metcalfe and Chalk (1950) and Assemein (1963) described the aspects of mature epidermis in a number of rubiaceous plants. The leaves of some species of Borreria, Diodia, Galium, Litorella, Rubia and Spermacoce are amphistomatic according to these authors. Pant and Mehra (1965) have observed stomata in the upper surface of the leaves of Borreria hispida, Gardenia gummifera and Paederia foetida. Bir Bahadur et al (1971) have reported amphistomatic leaves in 13 species. In addition to this
stomata have been observed on the upper surface of the leaves of *Crucianella angustifolia*, *Gardenia resinifera*, *Oldenlandia corymbosa*, *Pentas lanceolata* and *Sherardia arvensis* during the present studied. As regards, the mature aspect of the epidermis, the present observations are in accordance with those of Metcalfe and Chalk (1950).

The reports of occurrence of chloroplasts in the subsidiary and epidermal cells by Pant and Mehra (1965) and Bir Bahadur et al (1971) has been confirmed.

The structure and ontogeny of stomata have been studied in the Rubiaceae by Tognini (1897), Pant and Mehra (1965) and Bir Bahadur et al (1971). Tognini (1897) reported typical rubiaceous stomata in *Coffea arabica*. Pant and Mehra (1965) studied the development of stomata in 14 species of the Rubiaceae. These authors reported typical mesogene paracytic (rubiaceous) stomata but in addition reported amisocytic and stomata with a single subsidiary cell. Bir Bahadur et al (1971) studied structure, development and distribution of stomata in 26 species of the family. They confirmed the results of Pant and Mehra (1965) and pointed out that in organs other than leaves anomocytic stomata occur. These authors reported paracytic, anomocytic, anisocytic and diacytic stomata in *Hamelia patens*. The present observations agree with those of Bir Bahadur et al (1971), but, here the diacytic stomata have been rarely observed in the leaves of *Asperula arvensis*. Bir Bahadur et al (1971) have also given taxonomic significance to stomata.
The anomocytic stomata are perigenous and the paracytic, anisocytic and diacytic stomata are mesogenous in their development. The paracytic stomata are monocyclic, incompletely or completely amphicyclic. The abnormalities observed are arrested development, single guard cell with or without pore, contiguous stomata, division of guard cell and persistent guard mother cell.
### Table

#### STOMATA

<table>
<thead>
<tr>
<th>Name of the plant/organ</th>
<th>Anotis foetida</th>
<th>Anthocenhalus indicus</th>
<th>Asperula arvensis</th>
<th>Cinchona officinalis</th>
<th>Coffea arabica</th>
<th>C. e.1unoides</th>
<th>Crucianella angustifolia</th>
<th>Dentella repens</th>
<th>Galium odoratum</th>
<th>G. palustris</th>
<th>G. rubiensis</th>
<th>G. tricorneae</th>
</tr>
</thead>
<tbody>
<tr>
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<td>96</td>
<td>53.5</td>
<td>35.1</td>
<td>109</td>
<td>76.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of guard cells in μ</td>
<td>L</td>
<td>19</td>
<td>37</td>
<td>26.4</td>
<td>42.0</td>
<td>25.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of epidermal cells in μ</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Nature of epidermal walls</td>
<td>Hypostomati</td>
<td></td>
<td>Sinuous</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>Amphistomat</td>
<td></td>
<td>Straight &amp; arched</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of guard &amp; epidermal cells in μ²</td>
<td>L</td>
<td>19</td>
<td>37</td>
<td>26.4</td>
<td>42.0</td>
<td>25.5</td>
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<td></td>
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<tr>
<td>Size of epidermal cells in μ²</td>
<td>B</td>
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<td>Hypostomati</td>
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<td>Sinuous</td>
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<td>Surface</td>
<td>Amphistomat</td>
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<td>Straight &amp; arched</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### EPIDERMIS

- Frequency per mm²
- Size of cells in μ
- Nature of wall
- Size of guard cells in μ

**Note:** Showing percentage of different types of stomata, stomatal frequency and index per mm², size of guard & epidermal cells in μ, nature of epidermal walls and Surface.
Table: (Fig. 1:2, A, O = 425X; B-N, P, R = 350X; R = 230X)

Showing percentage of different types of stomata, Stomatal frequency and index per mm², Size of guard & epidermal cells in μ, nature of epidermal walls and Surface.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the plant/organ</th>
<th>STOMATA</th>
<th>EPIDERMIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percentage</td>
<td>Frequency per</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P. A. A.</td>
<td>mm²</td>
</tr>
<tr>
<td>A-C.</td>
<td>Gardinea resinifera</td>
<td>100 - -</td>
<td>624</td>
</tr>
<tr>
<td>D.</td>
<td>Hamelia patens</td>
<td>100 - -</td>
<td>554</td>
</tr>
<tr>
<td>E.</td>
<td>Hymenodictyon excelsum</td>
<td>100 - -</td>
<td>256</td>
</tr>
<tr>
<td>F-G.</td>
<td>Ixora coccinea</td>
<td>100 - -</td>
<td>464</td>
</tr>
<tr>
<td>H.</td>
<td>I. javanica</td>
<td>100 - -</td>
<td>416</td>
</tr>
<tr>
<td>I.</td>
<td>I. parviflora</td>
<td>100 - -</td>
<td>496</td>
</tr>
<tr>
<td>J.</td>
<td>I. stricta</td>
<td>100 - -</td>
<td>416</td>
</tr>
<tr>
<td>K.</td>
<td>Mitragyna parviflora</td>
<td>100 - -</td>
<td>368</td>
</tr>
<tr>
<td>L.</td>
<td>Morinda citrifolia</td>
<td>100 - -</td>
<td>320</td>
</tr>
<tr>
<td>M-N.</td>
<td>M. tinctoria var. tomentosa</td>
<td>100 - -</td>
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<tr>
<td>O-P.</td>
<td>Mussaenda frondosa</td>
<td>98 1 1</td>
<td>624</td>
</tr>
<tr>
<td>Q-R.</td>
<td>Oldenlandia corymbosa</td>
<td>94 6 -</td>
<td>496</td>
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</table>

| No. | | | | | | | | | |
Table: (Fig.1:3; B = 425X; A-C,G,I-J,O-P = 350X; H,K-N = 230X)

Showing percentage of different types of stomata, Stomatal frequency and index per mm², Size of guard & epidermal cells in μ, nature of epidermal walls and Surface.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the plant/organ</th>
<th>STOMATA</th>
<th>EPIDERMS</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Percentage Paracytic</td>
<td>Frequency per mm²</td>
</tr>
<tr>
<td>A</td>
<td>Pentas carnea</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>B-D</td>
<td>P. lanceolata</td>
<td>100</td>
<td>480</td>
</tr>
<tr>
<td>E</td>
<td>P. lanceolata var.</td>
<td>100</td>
<td>480</td>
</tr>
<tr>
<td>F</td>
<td>Rondeletia odorata</td>
<td>100</td>
<td>336</td>
</tr>
<tr>
<td>G</td>
<td>Rubia cordifolia</td>
<td>100</td>
<td>192</td>
</tr>
<tr>
<td>H-J</td>
<td>Sherardia arvensis</td>
<td>100</td>
<td>464</td>
</tr>
<tr>
<td>K-L</td>
<td>Barreria articularis</td>
<td>100</td>
<td>128</td>
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<td>B. stricta</td>
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<td>272</td>
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<tr>
<td>O-P</td>
<td>Xeromphis spinosa</td>
<td>100</td>
<td>368</td>
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</table>
PLATE - 1:1
EXPLANATION OF PLATE FIGURES (1:1 A - H X 880)
EPIDERMAL PEEL FROM LOWER SURFACE SHOWING:-

A. Galium odoratum:-
   Paracytic stoma and two meristemoids.

B - D. Asperula arvensis:-
   B. Diacytic like stoma.
   C-D. Contiguous stomata.

E. Galium tricoranae:-
   Arrested development.

F. Galium rubioides:- Division of one of the guard cells
   Division of one of the guard cells.

G. Crucinela angustifolia:-
   Single guard cell.

H. Asperula arvensis:-
   Persistent stomatal initial.