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STUDIES ON NORMAL AND ABNORMAL FOLIAR STOMATA IN THE SEEDLING OF SOME APOCYNACEAE

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Anomocytic, haplocytic, paracytic and anisocytic stomata are observed in the leaves of the seedlings of eight species of Apocynaceae. Their structure, development and distribution in different species are studied. Various types of stomatal abnormalities, juxtaposed stomata, superposed stomata, stoma with single guard cell, stoma without guard cell and divisions of guard cells and subsidiary cells are also observed. Cytoplasmic connections are found between the two guard cells of different stomata. Rarely a guard cell is not found associated with any pore.

Keywords : Apocynaceae; Seedlings; Stomata.

Introduction

The information about the stomata of Apocynaceae is scanty. Metcalfe and Chalk[1] and Patel *et al.*[2] described the structure and types of mature stomata. The present work is the study of the development of normal and abnormal stomata in the first formed leaves of the seedlings of *Adenium obesum* Roem. & schult., *Carissa carandas* L., *Cascabela thevetia* (L.) Lippold., *Catharanthus roseus* (L.) G. Don., *Holarrhena pubescens* (Buch. - Ham.) Wallich ex G. Don., *Nerium oleander* L., *Rouvolfia tetraphylla* L. and *Wrightia orborea* (Dennst.) Mabb.

Materials and methods

The epidermal peels were taken from the adaxial and abaxial sides of leaves with the help of sharp razor blade, needle and fine forceps. Sometimes epidermal peels were obtained treating desired pieces of the leaf with 30- 40% HNO_3. The peels were stained in Delafied's haematoxylin and counterstained with safranin and mounted in glycerine / glycerine jelly.

Observations

The leaves are hypostomatic except in *Catharanthus roseus*, where they are amphistomatic. In the leaves of *Nerium oleander* the stomata are sunken in a cavity and covered by many unicellular trichomes.

Normal stomata :- Anomocytic (Fig. 1 A), haplocytic (Fig. 1 B), paracytic (Fig. 1 C) and anisocytic stomata (Fig. 1 D) are observed in all species except *Nerium oleander* where anisocytic stomata are not found.

The development of stomata occurs from oval (Fig. 1 E), or tetrangular meristemoid (Fig. 1 F). Triangular meristemoid is rare and observed only in the leaves of *Catharanthus roseus* and *Holarrhena pubscens* (Fig. 1 G). It is smaller in the size and contains dense protoplasm. For the development of anomocytic stomata, it undergoes transverse or diagonal division to form two equal guard cells, which are surrounded by normal epidermal cells. (Fig. 1 A, H).

The haplocytic stomata are produced by two divisions of meristemoid (Fig. 1 I, J). When both divisions are parallel, a haplocytic stoma with lateral subsidiary cell is formed (Fig. 1 B). Occasionally these two divisions occur almost at right angle to form haplocytic stomata with a polar subsidiary cell (Fig. 1 K, L). Sometimes the second division occurs obliquely in such a way that the subsidiary cell remains partly polar and partly lateral in position (Fig. 1 M)

For the development of anisocytic stomata a meristemoid divides by a slight curved wall to form two unequal cells (Fig. 1 N). The larger cell differentiates as the first subsidiary cell while smaller one divides by wall perpendicular to the first division to form a second subsidiary cell and a middle triangular cell (Fig. 1 O) which divides on its third side to form a third subsidiary cell and a small central cell (Fig. 1 P). Latter divides to form two guard cells. Thus a

Fig. 1. Studies on normal and abnormal foliar stomata in the seedlings of some Apocynaceae.

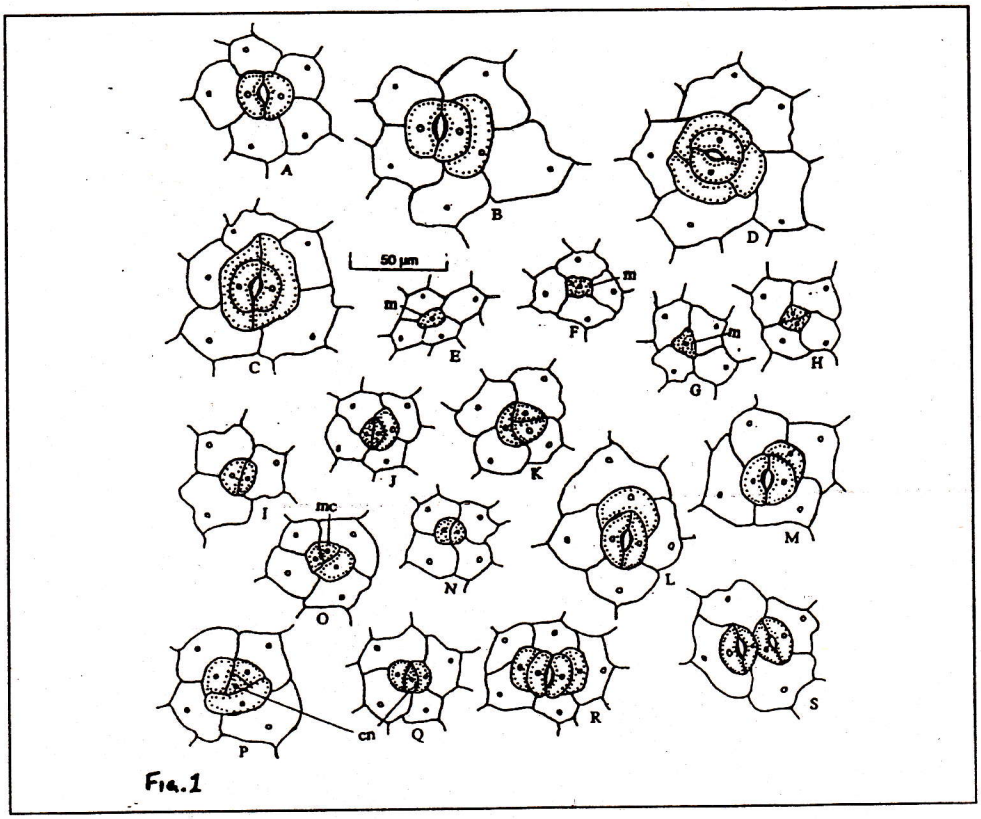

Fig. 1A-S : Mature and developmental stages of stomata. A, anomocytic Stomata; B,L,M, haplocytic stomata; C, R, paracytic stomata; D, anisocytic stomata; E, F, G, meristemoid; H, divided meristemoid; I, J, K, developmental stages of haplocytic stomata; N, O, P, developmental stages of anisocytic stomata; Q, developmental stages of paracytic stomata; S, juxtaposed contiguous stomata.

cn - central cell; m- meristemoid; mc- middle cell.

(A,E,H,S, *Catharanthus roseus;* B,K,L, *Carissa carandas;* C,G,Q, *H. pubescences;* D,I,J,N,O,P, *A. obesum;* F, *R. tetraphylla;* M, *N. oleander; R, W. arborea.)*

stomata with three unequal subsidiary cells are formed (Fig. D).

The development of paracytic stomata is slightly different from anisocytic stomata where meristemoid divides twice to form a smaller central cell and two larger cells. Latter differentiate as two subsidiary cells (Fig. 1 Q). The central cell divides to form two guard cells. A stoma developed in this method has two laterally arranged subsidiary cells, parallel to the longer axis of guard cells. They either completely surround the guard cells as in the leaves of *Holarrhena pubescens, Nerium oleander* and *Rauvolfia tetraphylla* (Fig. 1 C) or restricted to the lateral region of the guard cells. The polar regions are flanked by epidermal cells (Fig. 1 R). Such paracytic stomata are found in *Adanium obseum, Carissa carandas, Cascabela thevetia, Catharanthus roseus* and *Wrightia arborea.*

Abnormal stomata

Contiguous stomata : Juxtaposed contiguous stomata are observed in *Adanium obesum, Carissa carandas, Cascabela thevetia, Catharanthus roseus, Holarrhena*

Fig. 2. Studies on normal and abnormal foliar stomata in the seedlings of some Apocynaceae.

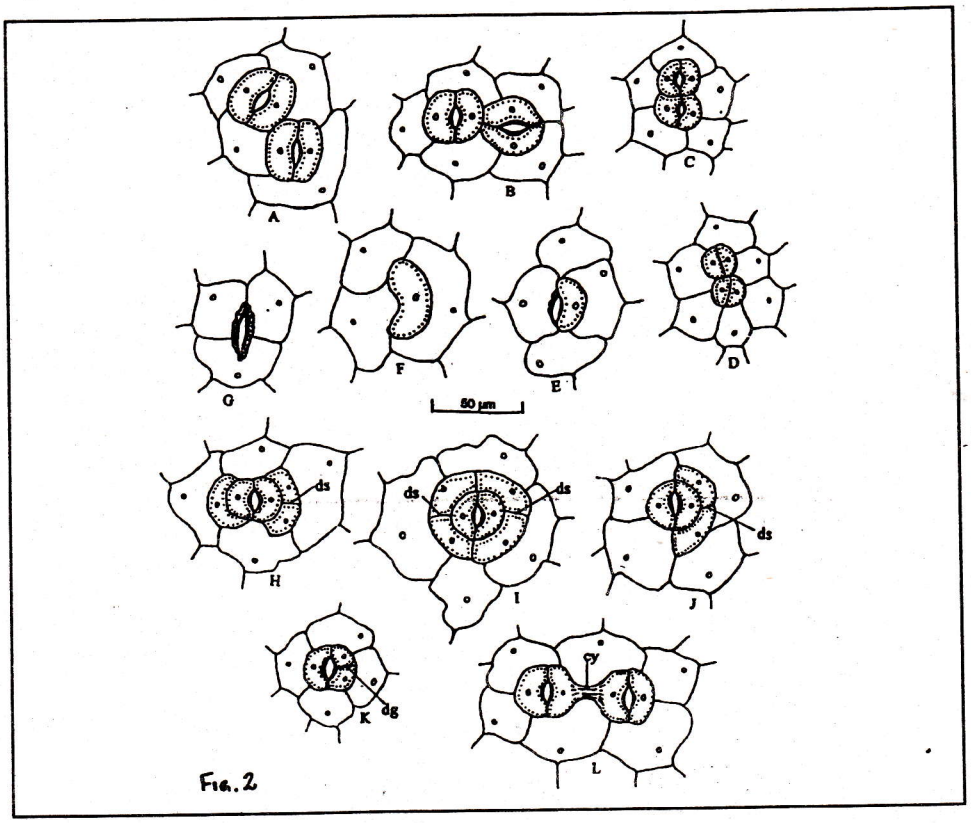

Fig. 2A-L : Mature and developmental stages of stomata. A,B, juxtapose contiguous stomata; C, superposed stomata; D, divided twin meristemoids; E, stoma with a single guard cell; F, a single guard cell; G, stoma without guard cell; H-J, stoma with divided subsidairy cells; K, stoma with divided guard cell; L, stomata with cytoplasmic connection.

cy - cytoplasmic connections; dg - divided guard cell; ds - divided subsidiary cells.

(A, L, *H. pubescens;* B, *W. arborea;* C, *N. oleander;* E, F, *Catharanthus roseus;* G, I, *Cascabela thevetia;* J, K, *R. tetraphylla.)*

pubescens, Nerium oleander and *Rauvolfia tetraphylla* (Fig. 1 S). Sometimes they are obliquely placed juxtaposed as in *Adanium obesum* and *Holarrhena pubescens* (Fig. 2 A). Rarely contiguous stomata arranged at right angle are also observed in *Adanium obesum* and *Wrightia arborea* (Fig. 2 B).

Superposed contiguous stomata are observed at or near their poles (Fig. 2 C). They are not common and found in *Adanium obesum, Carissa caranda* and *Nerium oleander.*

The development of contiguous stomata takes place from twin meristemoids (Fig. 2D).

Stoma with a single guard cell : Usually a stoma has two guard cells but in certain cases one guard cell is associated with a pore. Such guard cell form stomata with one guard cell. They are found in *Catharanthus roseus, Adanium obesum, Cascabela thevetia, Holarrhena pubescens, Nerium oleander, Rauvolfia tetraphylla* and *Wrightia arborea* (Fig. 2 E). Formation of such stoma is due to degeneration of one of the guard cells. During the degeneration gradual loss of contents occurs, size reduces and finally get compressed along the pore (Fig. 2E).

Rarely a guard cell is not found in association with any pore in *Catharanthus roseus* (Fig. 2 F). Therefore it is designated as a single guard cell and not a single guard celled stoma.

Stoma without guard cell : A pair of guard cells degenerates after the formation of pore in *Cascabela thevetia, Rauvolfia tetraphylla* (Fig. 2 G). The persistant pore can be designated as stoma without guard cells.

Division of subsidiary cells : Sometimes increase in the number of subsidiary cells occurs due to their divisions. In *Adanium obesum, Cascabela thevetia, Holarrhena pubescens* and *Rauvolfia tetraphylla*, one or both laterally located subsidiary cells in paracytic stomata divide parallel to the longer axis of the pore to form three or four subsidiary cells (Fig. 2 H, I). Similar division is also observed in the subsidiary cell of a haplocytic stomata in *Rauvolfia tetraphylla* to form two subsidiary cells (Fig. 2 J).

Division of guard cell : Rarely a guard cell divides perpendicular to the longer axis of the pore to form two shorter guard cells as in *Rauvolfia tetraphylla* (Fig. 2 K). This forms a stoma with three guard cells.

Cytoplasmic connections : Cytoplasmic connection are found between the two guard cells of two different stomata. They are not common and found in *Catharanthus roseus, Holarrhena pubescens* (Fig. 2 L).

Discussion

According to Metcalfe and Chalk[1] in members of family Apocynaceae either ranunculaceous (anomocytic) type or rubiaceous (paracytic) type of stomata are present. Patel *et al.*[2] have reported one more type cruciferous (anisocytic) type of stomata in *Aganosoma dichotama.* In addition to this haplocytic stomata are also observed in the present work.

According to Ahmad[3] and Patel and Shah[4] the abnormalities like stoma with one guard cell are due to degeneration of guard cell/cells. The present study agree with the observations of Ahmad[3] and Patel and Shah[4]. Patel *et al.*[2] reported two types of single guard cells; with pore and without pore in *Agonosoma dichotoma* and considered the single cells with pore as stomata with one guard cell and those without pore as single guard cells. The presence of types of guard cells, associated with or without pore in the seedlings of Apocynaceae support the observations and view of Patel *et al.*[2]. Gertz, Kuster and Guttenberg (cf. Dehnel)[5] have explained the division of guard cells induced under pathological conditions. Patel *et al.*[2] reported guard cells dividing under normal conditions in *Aganosoma dichotoma.* In the present work also the divisions of guard cells are found in normal conditions.

Cytoplasmic connections between the guard cells of nearby stomata have been observed in the leaves of *Catharanthus roseus* and *Holarrhena pubescens* which are not reported in Apocynaceae by earlier workers. Liz and Kimmins[6] are of opinions that cytoplasmic connections serve as pathways for the exchange of materials.

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