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Multiplicity of Stomatal Types in *Calotropis procera* R. Br.

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Organisation and ontogeny of stomatal apparatus have been investigated in *Calotropis procera.* The leaves are amphistomatic; the stomata are oriented irregularly and show marked variations in their organization and configuration, belonging to para- (most common), cyclo-, aniso-, and tetracytic types. All the three modes of ontogeny have been observed (mesogenous for paracytic and anisocytic; mesoperigenous for cyclocytic and perigenous for tetracytic stomata). The stomatal size also varies – the cyclocytic type being largest of all.

Introduction

That the stomatal apparatus shows variations in the organization and ontogeny at the family and generic level, has been recorded for the past more than five decades now. However, *Calotropis procera*, an evergreen, xerophytic species of the Asclepiadaceae shows several variations in these features on one and the same surface of a leaf. It, therefore, forms the subject matter of the present paper.

Materials and Methods

Leaves were collected from the Delhi University Campus as well as along the banks of the river Alaknanda in the Garhwal region. Portions of mature leaves (7th and 8th leaves from the apex) were fixed in FAA and later preserved in 70% ethyl alcohol. Epidermal strips were peeled off, stained with Delafield's haematoxylin, and mounted in 50% glycerine. To study the stomatogenesis, newly formed and unfolded young leaves were fixed in Carnoy's solution and subsequently stored as above. In the latter case, staining was done with acetocarmine and mounting in canada balsam after passing through a gradual series of n-butyl alcohol for dehydration.

Observations

Mature Stomata: The cells of both the epidermal surfaces are polygonal with slightly curved walls and show cuticular striations (figure 1 P). The leaves are amphistomatic with irregularly distributed stomata. They exhibit variable configuration and organization (figure 1 A, B). Majority of the stomata are paracytic on both the surfaces. However, cyclocytic, tetracytic, and anisocytic stomata are also commonly interspersed in between (figure 1A–L). Besides these, anomocytic and para-allelocytic types are but very rarely encountered (figure 1M, E), and these may be treated as aberrations. Also, juxtaposed contiguous stomata and stomata with a single guard cell occur as abnormalities (figure 1 N, O). It is important to mention that the cyclocytic, tetracytic, and anisocytic stomata are fewer on the upper surface in comparison to those on the lower one whereas the anomocytic and para-allelocytic are totally absent from the upper surface. The size of different stomatal types is also variable. On an average, the cyclocytic stomata (21.4×34.0 μm) are larger than the other types (tetracytic—18.6×26.4 μm and paracytic — 19.2×26.4 μm). Anisocytic stomata are of medium dimensions (20.0×25.6 μm). Usually the paracytic stomata on the upper surface are found to be smaller (14.8×21.3 μm) than those of the lower surface (19.2×26.4 μm). These possess one, two or rarely three subsidiary cells on either side of the two guard cells, placed in a parallel pattern (figure 1 C, D). In some instances, only one or two subsidiary cells have been seen on one side and two or three on the other. Divisions in them result in the formation of four or five subsidiary cells on one side and a single cell on the other. These are smaller and usually become arranged in two or three tiers, two cells in each tier para-allelocytic type (figure 1 E). In the cyclocytic stomata, the guard cells are at first encircled by 4–7, equal (figure 1 J) or usually unequal subsidiary cells, with a pair of superimposed laterals on either side (figure 1 K) or which are again surrounded by another circle of 7–12 cells, either in one or two rings. The cells of the outer ring (s) are markedly smaller than those comprising the inner one (figure 1 F). In figure G these cells present an irregular size. Tetracytic stomata which are less common than the cyclocytic ones, exhibit four subsidiary cells around the two guard cells, two laterals and two polars. These may or may not be equal in size (figure 1 H, I). The anisocytic stomata which generally possess three subsidiaries of different size are seen in figure 1 L. The anomocytic stomata are in contact of 6–8 ordinary epidermal cells (figure 1 M).

Table 1 shows the stomatal indices and frequencies of the various types of stomata on both the upper and lower leaf surfaces.

Stomatogenesis: The meristemoids (M) or the stomatal initials lie scattered all over the protodermal tissue and can be distinguished from other cells of this tissue by their lenticular shape, dense cytoplasm, prominent nucleus, and deeper staining behaviour (figure 2 A). The further development of the four types of stomata is as follows.

During the initiation of a paracytic stomata, the meristemoid divides unequally and a large and a smaller cell are produced (figure 2 B). Of these the larger one usually does not divide further but differentiates into the first subsidiary cell (S_1). On the other hand, the smaller cell divides by a curved wall to produce two unequal cells once again. The larger of these becomes the second subsidiary cell (S_2). The smaller one—the guard-cell-mother cell—enlarges and divides perpendicular to the

Table 1 *Variations in the stomatal types and stomatal frequency in* Calotropis procera

Leaf surface	Stomatal Indices	Paracytic stomata	Cyclocytic stomata	Tetracytic stomata	Anisocytic stomata
LOWER					
Base	6.97*	62	15	14	28
Middle	8.37	77	18	16	14
Apex	6.38	70	8	7	8
UPPER					
Base	7.18	84	8	—	—
Middle	7.29	80	6	6	4
Apex	7.59	96	—	—	—

* Average of 27 values

earlier divisions thus giving rise to two identical guard cells (figure 2 C, D). Usually the subsidiary cells do not divide. If at all they do so, their number is variable and depends upon the divisions which are always vertical. As such the subsidiary cells are always arranged parallel to the long axis of the guard cells (figure 2 F, G). Thus there may be two or three subsidiary cells (sometimes this number may be even more) located on either side of the guard cells. In some instances, one subsidiary cell of the paracytic stomata does not divide, whereas the second undergoes a vertical or transverse division. One thus notices four or five cells in two tiers—two on each side. This condition results in the development of the para-allelocytic stomata. Since the subsidiary cells as well as the guard cells originate from the same initial, the development conforms to the syndetocheilic type of Florin (1931, 1958), mesogenous type of Pant (1965) or sahodar sahkoshik type of Paliwal (1969).

The development of the cyclocytic stomata also begins with the unequal division of the meristemoid which produces two unequal cells (figure 2 H). The larger of these becomes the subsidiary cell. On the other hand, the smaller cell, which is lenticular in outline, soon divides unequally by a curved wall (figure 2 I). The larger cell differentiates into the second subsidiary cell whereas the smaller divides unequally once again and gives rise to the third subsidiary cell, along its third cutting face (figure 2 J). The central cell, which becomes considerably smaller by now, acts as the guard-cell-mother cell, and divides vertically to form the two guard cells (figure 2 M). Meanwhile, one, two or all the subsidiary cells also divide in different planes and thus increase the number of subsidiary cells in the first ring. Simultaneously, the epidermal cells adjacent to the meristemoid divide irregularly around the developing stomata and thus give rise to the second and third ring (if present) of subsidiary cells (figure 2 M, N). These are much smaller than those produced by the meristemoid itself and some of them take part in the formation of the first ring of subsidiary cells also. In view of this, in a young stomatal apparatus of the cyclocytic type, the guard cells are distinctly surrounded by 5–7 subsidiary cells in the first ring and 7–12 cells in the

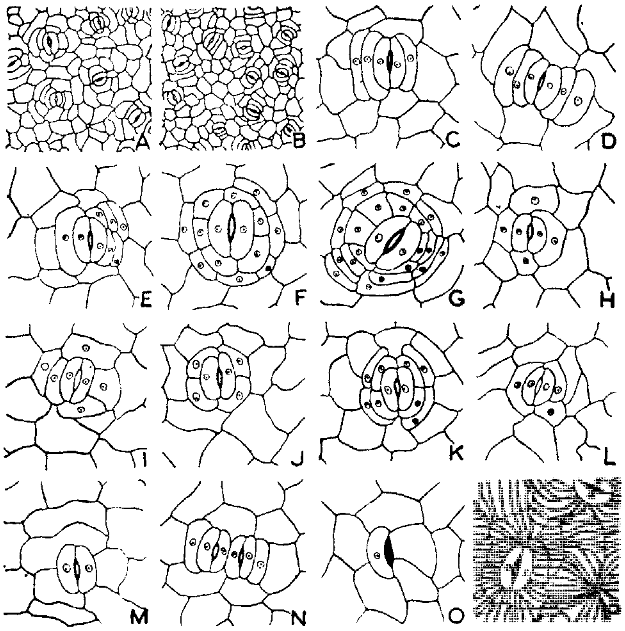

Figure 1

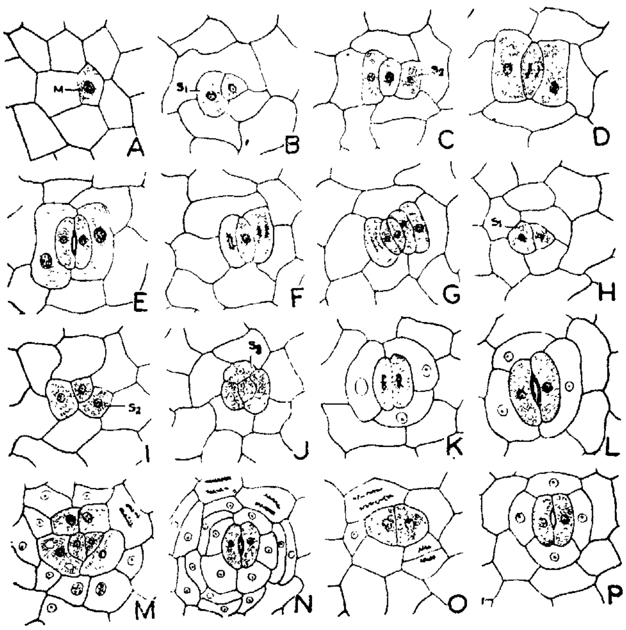

Figure 2

second and third rings, respectively, being composed of cells of variable shapes and sizes. The ontogeny, therefore, reveals that the 12–19 cells surrounding each pair of guard cells in a mature stomatal apparatus have a dual origin. Accordingly, the development conforms to the mesoperigenous category. The development of the tetracytic stomata begins with the division of the meristemoid which results in the formation of two equal-sized guard cells (figure 2 O). Here it seems that the subsidiary cells are not produced from the meristemoid; instead the surrounding epidermal cells divide simultaneously with the division of the meristemoid and its daughter cells, and arrange themselves around the young stoma (figure 2 O, P). The shape and size of the subsidiary cells thus produced may either be identical or may show alterations. Since the meristemoid here forms the two guard cells only but not the subsidiary cells, the mode of ontogeny falls under the perigenous (Pant 1965) or sahkoshik (Paliwal 1969) category.

The development of the anisocytic stomata follows a typically mesogenous pattern. The meristemoid divides by a curved wall and produces two unequal cells. The larger one gradually gets modified into the first subsidiary cell. The smaller divides once again unequally and produces the second subsidiary cell. Another similar division of the smaller cell results in the formation of the third subsidiary cell (figure 2 H–J). At this stage, the three subsidiary cells surround a central, considerably smaller cell which now acts as the guard-cell-mother cell and divides vertically to form the two guard cells (figure 2 J–L). Among the three subsidiary cells, one is considerably smaller than the other two.

The points made out earlier make it abundantly clear that ontogenetically all the four types of stomata follow an identical pattern but the differences appear at later stages. For instance during the development of both the anisocytic and cyclocytic type of stomata the meristemoid possesses three cutting faces and behaves like the apical cell of the lower plants. Also to be remembered is the fact that in successive stages of development, it is always the smaller partner which retains the meristematic capacity.

Discussion

As stated earlier, considerable variation has been recorded in the organization and ontogeny of the stomatal apparatus of *C. procera* so much so that most of the stomatal types recognized for the angiosperms (on a broad basis) are met with on the same leaf surface. Abnormalities such as stomatal apparatus with a single guard cell and contiguous

Figure 1 A–P Variations in the organization of stomata in *Calotropis procera*. *A*, Part of lower epidermis showing paracytic, cyclocytic, and tetracytic stomata (×100); *B*, Upper epidermis with paracytic as well as tetracytic stomata (×100); *C*, *D*, Paracytic stomata with unequal number as well as one pair of subsidiary cells on either side of the two guard cells (×230); *E*, Para-allelocytic stomata (×230); *F*, *G*, Cyclocytic stomata with two and three rings of subsidiary cells, respectively (×230); *H-K*, Tetracytic stomata with unequal and variable number and position of the subsidiary cells (×230); *L*, Anisocytic stomata (×230); *M*, Anomocytic stomata (×230); *N*, *O*, Contiguous stomata of the paracytic category; in 'O' a single guard cell is seen (×230); *P*, A few epidermal cells showing cuticular striations (×230)

Figure 2 A–P Stomatogenesis of various types of stomata. *A*, Meristemoid (*M*) (×230); *B-G*; Development of paracytic stomata *B-E* with a single and *F-G* with two subsidiary cells on either side of the guard cells (×230); *H-L*, Development of anisocytic stomata (×230); *M-P*, Ontogeny of the cyclocytic and tetracytic stomata (×230) stomata have also been encountered. A survey of the literature pertaining to the stomata reveals that occasional differences in the organization at mature stage on both the leaf surfaces and on one and the same leaf surface do occur (*see* Loftfield 1921, Sen 1958, Pant & Kidwai 1964, Paliwal & Kakkar 1968, Paliwal 1970, Kakkar & Paliwal 1974 and Sehgal & Paliwal 1974). In *Pereskia aculeata*, Paliwal and Kakkar (1968) observed considerable variations in the number of subsidiary cells around the two guard cells, ranging from 2–7, although majority of the stomata are of the paracytic type. Kakkar and Paliwal (1974) and Sehgal and Paliwal (1974) studied the various species of the genus *Euphorbia* and brought to light that this genus exhibits anomo-, aniso-, para-, and cyclocytic type of stomata or they may have a single subsidiary cell. Some species such as *E. antisiphylitica*, *E. boetica*, *E. cornuta*, *E. gerardiana*, *E. peplis*, and *E. rosea* show two or three types of stomatal apparatus on the same leaf surface. Evidently, such a variability in the organization of the mature stomata is arrived at either of the following patterns : (a) Differences in the number of subsidiary cells cut off from the meristemoid; (b) Divisions in the lateral ordinary epidermal or subsidiary cells produced by the meristemoid; (c) Divisions in the adjoining epidermal cells.

From the ontogenetic point of view, Tognini (1894) studied this aspect in 34 species of 29 dicotyledonous families and pointed out that different organs of a plant such as the cotyledons, leaves, perianth, and ovary wall sometimes exhibit variable patterns. Solereder (1908) stated that most of the families of the dicotyledons show more than one mode of development of the stomatal apparatus and the families Bixineae, Polygalaceae and Vochysiaceae exhibit as many as three patterns. Contrary to these observations, Stebbins and Khush (1961) concluded that "developmental modes are constant, even as to minute details, from organ to organ within the same plant". According to Paliwal and Bhandari (1962) the stomata in the outer integument of ovules, perianth lobes, and inner epidermis of pericarp in two species of Magnoliaceae follow the haplocheilic development whereas on the leaves they conform to the syndetocheilic type. In *Basella rubra* (Paliwal 1965) the stomatal apparatus may develop either in a partially haplocheilic manner with three or four subsidiary cells or in a syndetocheilic pattern with only two subsidiary cells. A similar situation exists in the taxon under examination here (*C. procera*).

From the above survey it is clear that even structurally different stomatal apparatus may have a similar ontogeny and contrarily structurally similar stomata may have a variable ontogeny. The latter condition is particularly true for the paracytic stomata of the Gramineae and Cyperaceae (*see* Campbell 1881, Porterfield 1937, Flint & Moreland 1946). Evidently, therefore, in order to obtain the true picture of the stomatal formation, developmental studies should be undertaken as also emphasized by Parkin (1924) and Maheshwari and Vasil (1961).

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