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Ontogeny of Normal and Abnormal Stomata in Seedlings of Some *Solanaceae*[1])

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With 34 Figures and 2 Plates

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Abstract

Seven types of aberrent and five types of normal stomata of the seedlings of 21 species of *Solanaceae* are discussed. The normal stomata are anomocytic, anisocytic, paracytic, diacytic and have a single subsidiary cell, with a haplocheilic or perigenous development in the first type and a syndetocheilic or mesogenous development in the other types. Anomalous developments noticed are: persistent stomatal initial which may be spherical or ovoid in surface view; single guard cell with or without pore; single guard cell looking like a stoma formed as a result of curvature and fusion of two ends; division of guard cell(s) through mitosis or due to ingrowth of guard cell wall; secondarily developed cytoplasmic connection between nearby stomata suggesting their physiological relation; aborted guard cells; and arrested development. Contiguous stomata develop either from adjacently placed meristemoids or as a result of readjustment during maturation. The variable behaviour of the meristemoid is also discussed.

Zusammenfassung

An Keimpflanzen von 21 Solanaceen-Spezies werden sieben Typen abnormaler und fünf Typen normaler Spaltöffnungsapparate beschrieben. Die normalen Stomata sind anomocytisch, anisocytisch, paracytisch und diacytisch oder mit nur einer Nebenzelle. Die anomocytischen Stomata

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entstehen haplocheil (perigen), die übrigen syndetocheil (mesogen). Als abnormale Bildungen werden festgehalten: in ihrer Entwicklung steckengebliebene, in Flächenansicht kugelig oder eiförmig erscheinende Stoma-Initialen, einzelne Schließzellen mit oder ohne Spalt oder infolge starker Krümmung und Verwachsung der Enden wie eine Spaltöffnung aussehend, Teilung der Schließzelle(n) durch Mitose oder durch Einfaltung der Zellwand; sekundär entstandene Plasmabrücken zwischen benachbarten Spaltöffnungen lassen auch physiologische Beziehungen zwischen diesen vermuten, schließlich verkümmerte Schließzellen und gehemmte Entwicklung. Einander berührende Spaltöffnungsapparate entstehen entweder aus eng beieinander liegenden Meristemoiden oder als Ergebnis einer Wiederherstellung während der Ausbildung der Epidermis. Das veränderliche Verhalten der Meristemoide wird besprochen. (Editor)

Introduction

INAMDAR & PATEL (1969) studied the development of foliar stomata in 12 species of the *Solanaceae*. They reported some stomatal abnormalities as did AHMAD (1964 a, b). Recently PATEL & INAMDAR (1971) studied the structure and ontogeny of stomata in 51 species of the *Polemoniales*, but the detailed development of abnormal types was not studied in these studies. DEHNEL (1961) studied the abnormal stomatal development in the foliage of *Begonia aridicaulis* ZIES., INAMDAR, GOPAL & CHOHAN (1969) and INAMDAR and PATEL (1970) described the development of normal and abnormal stomata in the foliage leaves of three species of the *Araliaceae* and *Plumbago zeylanica* respectively. We observed some anomalous develop-

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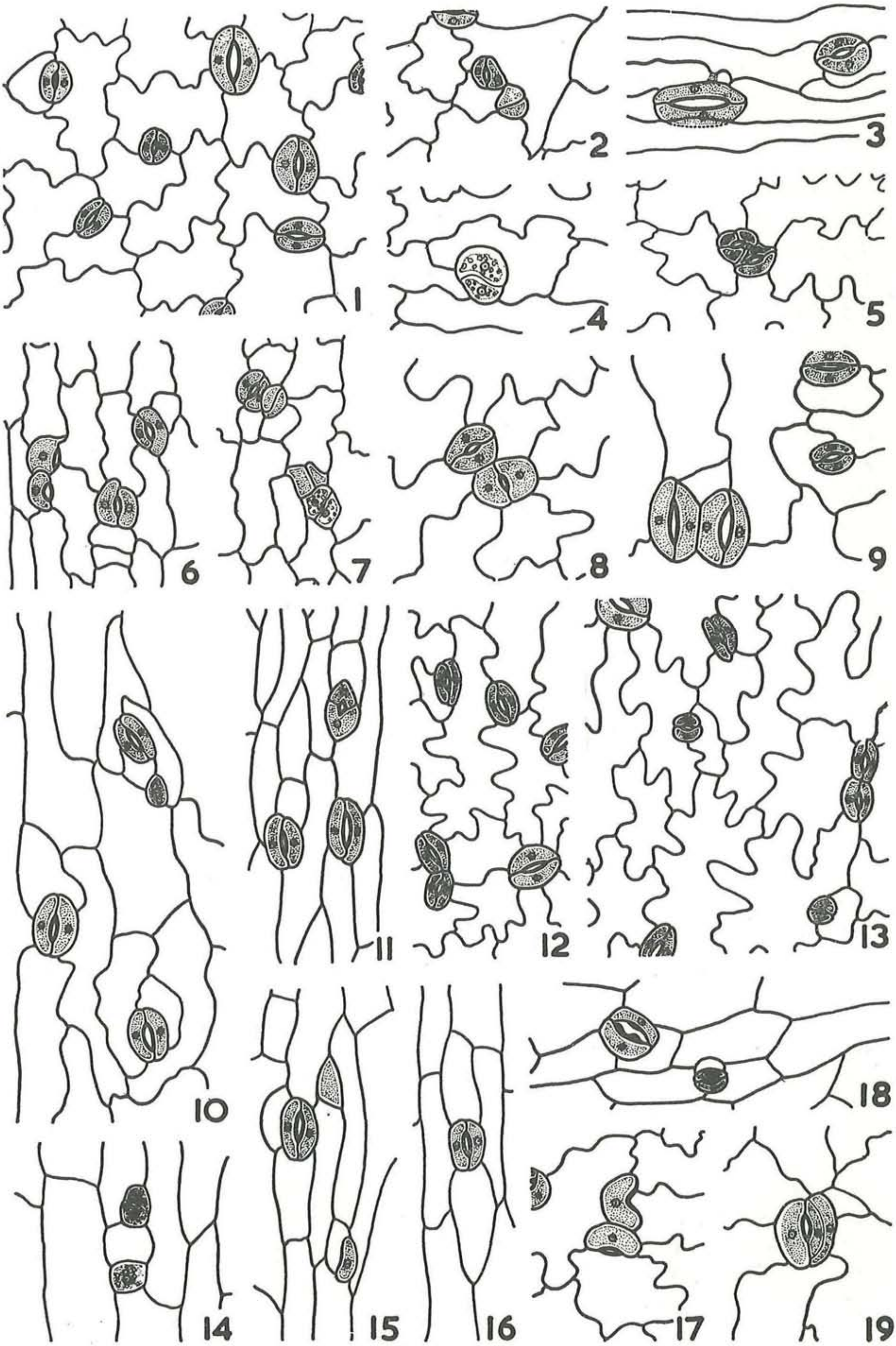
F'gs. 1—19. Epidermal peels showing normal and abnormal stomata (cotyledons all abaxial surface). 225x. — 1—2: *Browallia americana*, cotyledon: arrested development contiguous with normal stoma; — 3—4: *B. viscosa*, cotyledon: cytoplasmic connection (stippled) between a stoma and an epidermal cell (3) and division of persistent stomatal intial (4); — 5—7: *Datura gigantea*, cotyledon: division of guard cells (5), superimposed twin single guard cells (6), divided persistent stomatal initial and arrested development (7); — 8: *D. stramonium*, cotyledon: contiguous stomata; — 9—10: *Hyoscyamus niger*, cotyledon: contiguous stomata (9) and meristemoid (10); — 11: *Lycium ruthenicum*, hypocotyl: unequal guard cells; — 12: *Lycopersicon pimpinellifolium*, cotyledon: contiguous stomata; — 13: *Nicandra physaloides*, cotyledon: contiguous stomata; — 14—15: *N. physaloides* f. *alba*, hypocotyl: meristemoids (14), arrested development (strippled without nucleus) and single guard cell formation (15); — 17: *Nicotiana alata*, cotyledon: twin single guard cells; — 18: *N. sanderae*, hypocotyl: unequal thickening of cuticular ledges around the pore; — 19: *N. sylvestris*, cotyledon: longitudinal division in one of the guard cells.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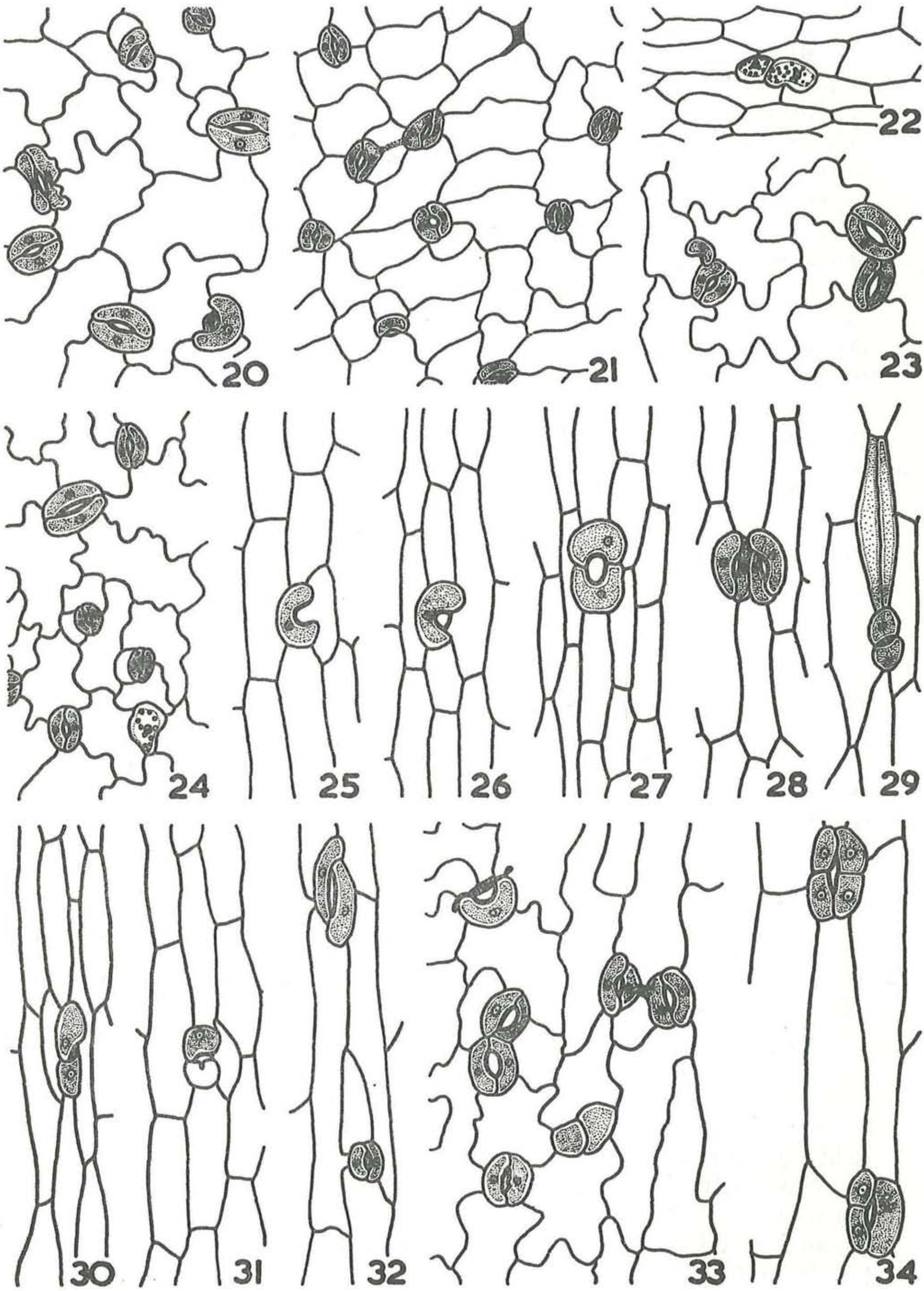
ments in the seedling stomata of 21 species of the *Solanaceae* and since it is a puzzle, decided to present an ontogenetic description of these types as it would be of interest to plant morphologists.

Materials and Methods

The seeds of the species investigated were obtained from the botanical gardens at Stockholm, Sweden and Berlin, West Germany through the courtesy of the directors. The seeds of the following 21 species were grown at an atmospheric temperature (April—42.2° C, May—44° C) in the Botanical Garden of Sardar Patel University, Vallabh Vidyanagar: *Browallia americana* L., *B. viscosa* H. B. K., *Datura gigantea* L., *D. stramonium* L., *Hyoscyamus niger* L., *Lycopersicon pimpinellifolium* (JUSL.) MILL., *Lycium ruthenicum* MURR., *Nicandra physaloides* (L.) GAERTN., *N. physaloides* f. *alba*, *Nicotiana alata* LINK et OTTO, *N. sanderae* Hort., *N. sylvestris* SPEG. et COMES, *Petunia axillaris* (LAMK.) B. S. P., *P. hybrida* Hort. ex DC., *Salpiglossis sinuata* HUI et PAV., *Schizanthus wisetenensis* Hort. ex GARD., *Solanum capsicastrum* LINK., and *S. dulcamara* L.

Epidermal peels were taken from fresh as well as fixed material (1 : 3 acetic-ethanol) of cotyledon and hypocotyl, stained with Delafield's haematoxylin and mounted in glycerin jelly by usual methods. Camera lucida drawings were prepared and series of photomicrographs were made of stained as well as unstained peels.

Figs. 20—34. Epidermal peels showing normal and abnormal stomata (cotyledons all abaxial surface except fig. 21). 225×. — 20: *Petunia axillaris*, cotyledon: single guard cell with pore and stoma with irregular outline; — 21: *Petunia hybrida*, cotyledon (upper surface): cytoplasmic connection between single guard cell with pore and aborted stoma; — 22: *idem*, hypocotyl: division of persistent stomatal initial; — 23: *P. nyctaginifolia*, cotyledon: single guard cell in relationship with normal stoma and contiguous stomata; — 24: *Physalis alkekengi*, cotyledon: persistent stomatal initial; — 25—28: *idem*, hypocotyl: single guard cell without pore (25) and with pore (26), stoma with abnormally large pore (27), contiguous stomata (28); 29—30: *P. ixocarpa*, hypocotyl: epidermal cell like arrested development (stippled without nuclei) and stoma with displaced and unequal guard cells; — 31: *Salpiglossis sinuata*, hypocotyl: note one of the guard cells without nucleus and cytoplasm with an ingrowth from the pore side; — 32: *Schizanthus wisetonensis*, hypocotyl: note stoma with displaced guard cells; — 33: *Solanum capsicastrum*, cotyledon: note an arrested development (stippled without nuclei), contiguous stoma, single guard cell with pore, and cytoplasmic connection between two guard cells; — 34: *S. dulcamara*, hypocotyl: note transverse division in the guard cells (oblique cell plate in lower stoma)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Observations

Mature Stomata: The cotyledons examined of all the species are amphistomatic. The epidermal walls are thick, being either sinuous, straight or arched (Figs. 1—34). The stomata are irregularly arranged on the cotyledon and parallel to the long axes or transversely or obliquely on the hypocotyl. Both normal and abnormal stomata may occur on the same surface of an organ. The normal stomata on the cotyledons and hypocotyl of investigated species are either anomocytic, anisocytic, paracytic, diacytic, or with a single subsidiary cell (Figs. 1—34). The mature stomatal apparatus consists of lenticular pore surrounded by two kidney-shaped guard cells. The guard cells may be equal or unequal and displaced (Figs. 30—32). This may be due to unequal division of the guard mother cell (Pl. II : L). Sometimes one of the guard cells is abnormally large so that it appears to encroach upon the other (Pl. I : F). Rarely the pore is also quite large and not lenticular (Fig. 27). The cuticular thickening around the pore is in most cases uniform and even, or less often uneven and not uniform (Fig. 18).

Several types of anomalous developments have been noticed both on the hypocotyl and cotyledons. In the species investigated by far the more common anomalies were the persistent stomatal initial (Figs. 4, 7, 22, 24; Pl. II : R—S); single guard cells (Figs. 6, 17, 20, 23, 26, 33; Pl. II : N—P, V); and division of guard cells (Figs. 5, 19, 34; Pl. I : G—I; Pl. II : K). The other abnormalities are single guard cells in relationship with normal stomata (Figs. 23; Pl. II : O—P); aborted guard cells (Fig. 21); arrested developments (Figs. 2, 7, 15, 33) and cytoplasmic connection between nearby stomata (Figs. 21, 33) or between stomata and epidermal cells (Figs. 3; Pl. I : J). On the foliage leaf surface of these species the anomalies are not so common. Mean values of 15 observations showing the size of epidermal cells and guard cells are given in the following Table 1.

Development: I. Normal (Pl. I: A—B)

a) Anomocytic stomata arise directly from the meristemoid by a straight division and without cutting off any subsidiary cells.

b) Paracytic stomata are formed by cutting off of two parallel subsidiary cells, one on either side of the meristemoid, and straight division of the guard mother cell parallel to the subsidiary cells.

c) Stoma with a single subsidiary cell arises after cutting off one subsidiary cell from the meristemoid, parallel to the guard mother cell.

d) Diacytic stomata arise if the guard mother cell divides by a wall at right angles to the subsidiaries.

e) Anisocytic stomata: Here the meristemoid behaves like an apical cell with three cutting faces, producing three subsidiary cells in a spiral fashion and then forming two equal guard cells by a straight division parallel to the last subsidiary cell.

Table 1

Average size of epidermal and guard cells (μ)

Species		Epidermal cells Length	Epidermal cells Breadth	Guard cells Length	Guard cells Breadth
Browallia americana	(C)	85	40	32	11
Browallia viscosa	(C)	99	42	37	10
Datura gigantea	(C)	93	28	31	10
Datura stramonium	(C)	90	31	42	8
Hyoscyamus niger	(C)	129	45	45	13
Lycium ruthenicum	(H)	108	17	39	14
Lycopersicon pimpinellifolium	(C)	67	32	31	9
Nicandra physaloides	(C)	97	30	29	9
N. physoloides f. *alba*	(H)	86	18	46	10
Nicotiana alata	(C)	113	48	36	10
Nicotiana sanderae	(H)	84	32	34	11
Nicotiana sylvestris	(C)	90	42	36	13
Petunia axillaris	(C)	86	44	36	13
Petunia hybrida	(C)	107	45	38	11
Petunia hybrida	(H)	117	21	37	11
Petunia nyctaginifolia	(C)	106	45	32	10
Physalis alkekengi	(C)	73	30	21	9
Physalis alkekengi	(H)	106	33	34	16
Physalis ixocarpa	(H)	135	21	39	14
Schizanthus wisetonensis	(H)	106	26	42	12
Salpiglossis sinuata	(H)	100	26	46	13
Solanum capsicastrum	(C)	93	35	40	13
Solanum dulcamara	(H)	165	34	47	21

(C) = Cotyledon, (H) = Hypocotyl

II. Abnormal Development

a) Persistant stomatal initial: The stomatal initial normally is devoid of chloroplasts, develops a uniform thickening, but lacks differential wall thickening. Chloroplasts appear later and become persistent (Figs. 4, 7, 22, 24; Pl. II: R—S). The persistent stomatal initial normally occupies the position of a stoma. It is either spherical (Pl. II: S) or ovoid (Figs. 22, 24) in outline, notched and sometimes shows protruberances (Pl. II: R). After the persistent stomatal initial is differentiated, it divides in any plane but an intervening pore doesnot develop (Figs. 4, 7, 22). It may occur solitary or in close association with arrested development (Fig. 7).

b) Arrested development: During ontogeny the nucleus and the cytoplasm of the stomatal meristemoids degenerate at any stage of development and become arrested (Figs. 2, 7, 15, 29, 33). Sometimes the arrested stomatal cells come to resemble the epidermal cells (Fig. 29). Arrested development can be easily distinguished from the persistent stomatal initial by lack of chloroplasts, a prominent nucleus and dense staining properties.

c) Single guard cell: The single guard cell (which for convenience may be termed half stoma) also arises directly from the meristemoid lacking chloroplasts. The meristemoid, instead of giving rise to a pair of guard cells, is notched on one side, chloroplasts appear and the cell becomes reniform or semilunar (Figs. 15, 20, 25—26; Pl. I: C; Pl. II: V). Here the differential wall thickening develops on the side of the notch and the pore is usually absent. The curvature sometimes is more pronounced in a half stoma, so that it forms a complete circle around the pore and fuses with the other end, looking like a stoma (Pl. II: M). The half stoma shows division (Pl. II: N), occuring either solitary or in close association and variable orientation with normal stomata (Fig. 23, Pl. II: O—P). In plate II: P, the half stoma appears like a basket over the top of a normal stoma. Rarely the meristemoid developing into a half stoma cuts off a small initial (Pl. I: D), which may develop into variously oriented twin half stomata (Figs. 6—7). The half stoma also arises as a result of degeneration of one of the guard cells of a normal stoma (Fig. 33, Pl. II: T).

d) Division of guard cell(s): The guard cell(s) of stomata both on hypocotyl and cotyledons mostly divide by transverse divisions (Figs. 5, 34, Pl. I: G—H; Pl. II: K) and rarely by longitudinal divisions (Fig. 19). The division of guard cell(s) is either due to mitosis and laying down of cell plates (Fig. 34) or may be due to ingrowth from the outer wall (Pl. II: L, U). Due to this division it appears as if the stoma is made up of three to four cells with a narrow (Pl. I: H; Pl. II: K, Q) or an abnormally large pore (Pl. I: I). An abnormally large pore is formed due to stretching of the guard cells.

We observed transverse, oblique, and longitudinal divisions of the guard cell/s in both the hypocotyl and cotyledons of most of the species investigated without subjecting the plants to any type of special conditions, in other words, the morphological modifications noticed here are not artificially induced. We have failed to observe proliferation of guard cells and formation of new stomata.

e) Cytoplasmic connection between nearby stomata: After the stomata are fully differentiated, sometimes a protruberence arises from one of the guard cells of a nearby stoma. The protruberences grow more and more, ultimately meeting and fusing in the center, their fusion wall disintigrated, and a communication channel is formed between two near by stomata. This tubular connection appears like a conjugation tube between two algal filaments. These guard cells have a cytoplasmic connection which is secondarily developed (Pl. I: J). We have also observed cytoplasmic connection between nearby placed stomata (Figs. 21, 33) and sometimes between one of the guard cells and an epidermal cell (Fig. 3). This indicates that there is some physiological connection between the nearby stomata and guard cell and an epidermal cell.

f) Degeneration of guard cells: Sometimes both guard cells degenerate and only a central thickening remains (Fig. 21). Degeneration of guard cells is commonly seen in mature leaves which are about to fall, but here it has been observed in seedlings which are yet to mature.

g) Contiguous stomata: Contiguous stomata are very common (Figs. 5, 8–9, 12, 13, 23, 28; Pl. I: E). They develop from the adjacently placed meristemoids or as a result of readjustment during maturation of the epidermis. The contiguous stomata have variable orientations, they may be juxtapposed, superimposed, obliquely oriented or at right angles to each other. In the literature contiguous stomata are regarded as an abnormality by several workers, but whether they are actually normal or abnormal can only be decided by studying their ontogeny, when they develop from two adjacently placed meristemoids or as a result of readjustment, we feel that they should not be regarded as abnormal, as there is a possibility here that the meristemoids are adjacently placed and the stomata have become contiguous during maturation of the epidermis.

The ontogeny of the anomocytic stomata resembles the haplocheilic type of Florin (1931, 1933) or perigenous type of Pant (1965), while that of the other types are syndelocheilic (Florin 1931, 1933) or mesogenous (Pant 1965).

Discussion

The occurence of aberrent stomatal developments in foliage leaves is by no means new and has been reported by several workers, but their relatively wide range observed in the seedling stomata of 21 species of the *Solanaceae* is perhaps unique.

According to our observations the persistent stomatal initial noticed here is not always a perfect circle and doesnot show attraction of nuclei of the adjacent cells. Ahmad (1964 a) also didnot observe attraction of nuclei of the adjacent cells by the persistent stomatal initial. The persistent stomatal initial may be spherical or ovoid in outline, notched, shows protruberances and also divides in any plane.

The guard cell division under induced pathological conditions has been discussed at length by Gertz (1919), Küster (1925, 1930) and Guttenberg (1905). Gertz (1919) induced several stomatal anomalies by growing seedlings of plants in various combinations of warm-light and dark-humid conditions. He managed to effect morphological modifications in a hypocotyl of *Cucurbita pepo* and in a cotyledon of *Luffa cylindrica*. Dehnel's (1957) attempts to induce guard cell division in *Begonia aridicaulis* and resumption of growth by artificial application of various chemical growth regulators were unsuccesful. According to KÜSTER (1930) division of guard cells occurs commonly in insect-induced galls. He also pointed out that there is no consistency of spindle-axis orientation during division as the wall may be oblique, transverse or parallel to the long axis of the guard cells. RAO & RAMAYYA (1967) reported proliferation of guard cells producing stomata on the connective of the anther in *Momordica charantia* L. and on the leaf of *Scaevola frutescens* KRAUSE.

The causes of such aberrent expression of morphological characters have been variously interpreted. According to KLEBS (1903, 1904) it may be due to "specific nature"; MORGON (1934) considered a "cytoplasmatic heterogeneity", MCCLINTOCK (1951, 1956) "gene action", BÜNNING (1952) "extrinsic factors", RAO & RAMAYYA (1967) "momentary developmental disturbances". INAMDAR, GOPAL & CHOHAN (1969) have pointed out that it is difficult to draw any definite conclusion. DEHNEL (1961) has negated the gene-controlled reaction and also pointed out that it is clearly not yet possible to determine whether extrinsic factors or intrinsic instability are responsible for stomatal ontogenetic aberrations in the lamina of *Begonia aridicaulis*. RAO & RAMAYYA (1967) have not given any causes for such developmental disturbances. There is little evidence to support the other two views.

By changing the external conditions GERTZ (1919), as pointed out earlier could induce stomatal abnormalities in the hypocotyl of *Cucurbita pepo*, and in the cotyledon of *Luffa cylindrica*. This is strong evidence in favour of BÜNNING's (1952) hypothesis. But when such stomatal anomalies occur in nature explanation becomes difficult. More difficult is the variable behaviour of the meristemoid on the same surface of an epidermis which gives rise to either a pair of guard cells, a persistent stomatal initial, a single guard ceil, arrested development or trichome (*see* BÜNNING 1952). From our studies we feel that such stomatal aberrations may not be due to one factor only. We obtained the seeds from Sweden and West Germany and they were grown in our Botanical Garden; so naturally the environmental conditions (extrinsic factors) were different from these countries. What induces the meristemoids on the same surface of an epidermis to behave in a variable way ? The possible answer which we can give from our studies is that the physiological conditions surrounding each meristemoid may be different or may be due to inhibitory substances, which may be responsible for abnormal stomatal development. Moreover, the cotyledons are the main source of food supply for the embryo during germination. The food is continuously absorbed from the cotyledons by the germinating embryo which might cause internal changes and might ultimately lead to stomatal aberrations. We believe that both the extrinsic and intrinsic factors might be involved in the abnormal stomatal development. However, we feel that experimental studies under aseptic and controlled conditions might serve to throw some light on this aspect of the problem.

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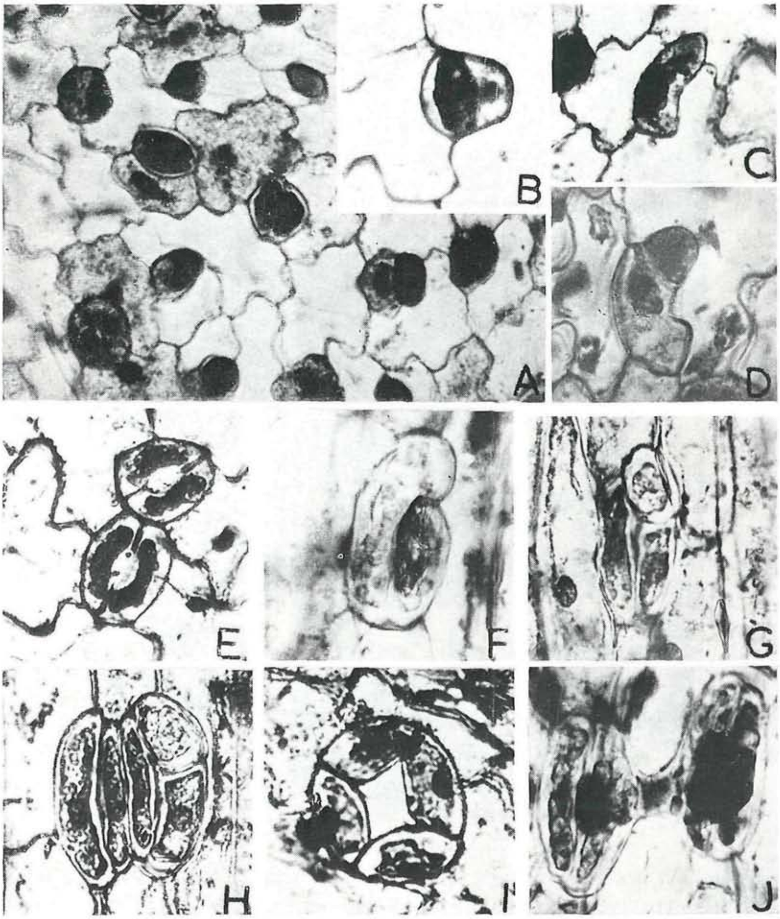

Datura stramonium, cotyledon: A: Meristemoids in stages of development (×720); — B: Formation of paracytic stoma (×550); — C: Formation of single guard cell directly from the meristemoid (×550); — D: Formation of single guard cells, note initial cut off at the end (×1100); — E: Contiguous stomata (×440);

Physalis ixocarpa (F—H: hypocotyl, I—J: cotyledon): F: Stoma with unequal guard cells and one encroaching guard cell (×370); — G—H: Division in one of the guard cells, note unequal and compressed guard cells in H (×370); — I: Stoma with four guard cells and a big pore formed as a result of transverse division in both the guard cells (×400); — J: Cytoplasmic connection between nearby stomata (×390).