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Effect of Growth Regulators on the Structure of Cotyledonary and Hypocotyledonary Stomata of *Gossypium herbaceum* var. Digvijay

By

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With 118 Figures (1 Plate)

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Abstract

Effect of certain growth regulators on the structure and ontogeny of stomata is described. The stomatal types observed are: anomocytic, with a single subsidiary cell, paracytic, diacytic, hemidiacytic, transitional between paracytic and diacytic and anisocytic. Developmental stages are observed for the first time in the dormant cotyledons. The development of anomocytic stomata is perigenous while that of other types is mesogenous. A new term "hemidiacytic" is suggested for a stoma which is enclosed by two subsidiary cells except on one side and whose common wall is at right angles to the guard cells. The aberrant types noticed in various treatments are: 2–3 anomocytic contiguous stomata, 2–3 anisocytic contiguous stomata, twin anisocytic stomata, twin paracytic stomata, one and a half contiguous stomata, anomocytic single guard cells, anisocytic single guard cells, paracytic single guard cells, degeneration of guard cells, division of guard cells, persistent stomatal cells, unusual thickening of the wall around the guard cells, obliquely oriented guard cells, cytoplasmic connection between adjacent stomata, stomata without pores, multinucleate guard cells, unequal guard cells and division of guard mother cell. Contiguous stomata develop in three ways: (i) from the adjacent meristemoids, (ii) one from guard mother cell and another from the smallest (third) subsidiary or its derivatives of an anisocytic stoma, (iii) from the derivatives of the smallest subsidiary cell of an anisocytic stoma. The development of twin anisocytic, twin paracytic, anomocytic single guard cell, paracytic single

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guard cell, anisocytic single guard cell and one and a half contiguous stomata is also described. On the basis of ontogeny the other new terminologies suggested are: anomocytic contiguous stomata, anisocytic contiguous stomata, twin anisocytic stomata, twin paracytic stomata, anomocytic single guard cell, paracytic single guard cell and anisocytic single guard cell.

Kurzfassung

Samen von *Gossypium herbaceum* L. var. Digvijay werden 20 Tage in Petrischalen auf täglich gewechseltem Filterpapier, das mit Lösungen von Wuchsstoffen getränkt wurde, keimen gelassen. Folgende Wachstumsregulatoren wurden geprüft: Gibberellinsäure, TIBA, IAA, Colchicin, Maleinhydrazid, Sulfanilamid in Konzentrationen 25, 50 und 100 ppm, ferner Rohrzucker 2000 ppm. Es wird eine große Zahl abnormer Stomatabildungen beschrieben, für die einige neue Termini vorgeschlagen werden; „hemidiazytisch" sind Spaltöffnungsapparate, deren Schließzellen von zwei Nebenzellen begleitet sind, deren gemeinsame Wand senkrecht zu einer Schließzelle steht und die das Schließzellenpaar nur einseitig umfassen (Fig. 27). Weitere Termini werden für Spaltöffnungszwillinge geprägt. Solche können auf dreierlei Weise entstehen: 1. aus benachbarten Meristemoiden, 2. ein Schließzellenpaar aus der Spaltöffnungsmutterzelle, das andere aus der kleinsten (dritten) Nebenzelle oder deren Abkömmling, 3. von den Abkömmlingen der kleinsten Nebenzelle einer anisozytischen Spaltöffnung.

(Editor compil.)

Introduction

Cotton is an important economic agricultural crop of India and especially Gujarat. Several varieties of cotton viz., Digvijay, Vijalp (2087), Kalyan, Sanjaya, 170 C.O.Z., Vishnu, I.S.C. cotton 67, Hybrid-4, Hybrid-5 and Varalaxmi are cultivated in Gujarat. Digvijay cotton is selected here because it does not require spraying of pesticides and insecticides.

Digvijay cotton (*Gossypium herbaceum* L.) is a member of the *Malvaceae*. Only a few reports exist on the epidermal structure and ontogeny of stomata in the *Malvaceae*. Inamdar & Chohan (1969a, 1969b) studied the epidermal structure and ontogeny of stomata in the vegetative and floral organs of *Hibiscus rosa-sinensis* L. and in the leaves of 15 species of the *Malvaceae* and 2 species of the *Bombacaceae* respectively.

The action of growth regulators on the structure and ontogeny of stomata of *Abelmoschus esculentus* is studied by Inamdar (1970). The other reports are those by Weber (1943), Tonzig & Ott-Candella (1946), Weissenböck (1949), Guyot (1964, 1970), Humbert & Guyot (1969) who studied the effect of colchicine on stomata. Recently Inamdar *et al.*

(1974), INAMDAR & GANGADHARA (1975) and GANGADHARA & INAMDAR (1975) reportet the effect of growth regulators on the cotyledonary stomata of some *Cucurbitaceae*.

Material and Methods

The seeds of *Gossypium herbaceum* L. var. digvijay used in the present study were brought from Agricultural farm, Surat. The fuzz was removed from the seeds with the help of a fine forceps. The graded seeds were surface sterilized with 5 vol.-% hydrogen peroxide. The surface sterilized seeds did not germinate well, but the unsterilized seeds germinated well. Hence, the unsterilized seeds were cultured in sterilized petridishes lined with sterilized filter papers under laboratory conditions (Temp. 18—26° C) using the following growth regulators as substrates: Distilled water (control, DW); Giberellic acid, Triiodobenzoic acid (TIBA), Indole-3-acetic acid (IAA), Colchicine (COL), Maleic hydrazide (MH), Coumarin (COU), Sulphanilamide (SUL), Morphactin (MOR), each substance in conc. 25, 50 and 100 ppm, and finally Sucrose (SUC) 2000 ppm. Adequate supply of substrates was maintained throughout the experiment. The petridishes and filter papers were renewed regularly once in 24 hours. The experiment was continued for 20 days till the complete maturation of cotyledons. The experiment was repeated for confirming the results.

Epidermal peels of young and mature cotyledons (lower) and hypocotyl were taken, stained in Delafield's haematoxylin, washed in distilled water and mounted in glycerin. Care was taken to depict the exact size, shape and position of nuclei and nucleoli while making camera lucida drawings.

Stomatal frequency, index, frequency of epidermal cells and size of guard and epidermal cells are compiled in Table 1.

Observations

The dormant cotyledons dissected out from the seeds were cleared and observed. The epidermal cells are polygonal, isodiametric with arched or slightly sinuous anticlinal walls. The developmental stages of stomata were observed (Fig. 1). However, the stomatal initiation was stimulated after the emergence of the radicle.

Mature epidermis: Hypocotyl: The epidermal cells are polygonal, and elongated in the direction of the long axis of the hypocotyl with straight or arched anticlinal walls. The hypocotyl is stomtic with stomata oriented either parallel, oblique or at right angles to the long axis of an organ (Figs. 3, 4, 21, 22).

Cotyledon: The epidermal cells are polygonal, isodiametric or elongated in various directions with straight, arched or sinuous anticlinal walls (Figs. 2, 5, 13, 110, 114). The cotyledons are amphistomatic. The

Table I

Showing stomatal frequency, Index; Frequency of epidermal cells per mm² Size of guard and epidermal cells in μm (Abbreviations see the text)

Treatments	Stomatal Frequency	Stomatal index	Size of guard cells		Frequency of epidermal cells	Size of epidermal cells	
			L [1])	B [2])		L [1])	B [2])
Control (DW)	240	13	17	7	1606	30	19
GA 25 ppm	227	15	17	7	1316	30	18
GA 50 ppm	266	16	18	7	1379	30	20
GA 100 ppm	275	17	19	8	1309	31	17
IAA 25 ppm	186	11	17	7	1467	28	16
IAA 50 ppm	269	16	18	7	1393	35	20
IAA 100 ppm	373	18	16	7	1621	31	17
TIBA 25 ppm	379	22	19	7	1376	40	17
TIBA 50 ppm	256	18	20	7	1170	34	19
TIBA 100 ppm	252	17	19	7	1329	43	23
SUC 2000 ppm	270	13	17	7	1856	34	14
COL 25 ppm	144	7	16	8	1861	30	16
COL 50 ppm	50	3	17	8	1749	32	17
COL 100 ppm	142	9	19	9	1621	34	15
MH 25 ppm	204	10	20	9	1964	41	16
MH 50 ppm	128	7	19	10	1803	39	17
MH 100 ppm	60	4	20	9	1558	40	15
COU 25 ppm	168	11	22	8	1312	42	16
COU 50 ppm	258	14	19	9	1622	42	15
COU 100 ppm	144	9	23	9	1488	40	15
SUL 25 ppm	307	16	23	11	1637	42	17
SUL 50 ppm	279	15	20	10	1648	41	17
SUL 100 ppm	263	16	24	11	1328	34	23
MOR 25 ppm	261	22	23	9	944	35	23
MOR 50 ppm	265	18	23	9	1222	36	20
MOR 100 ppm	314	20	24	10	1254	39	23

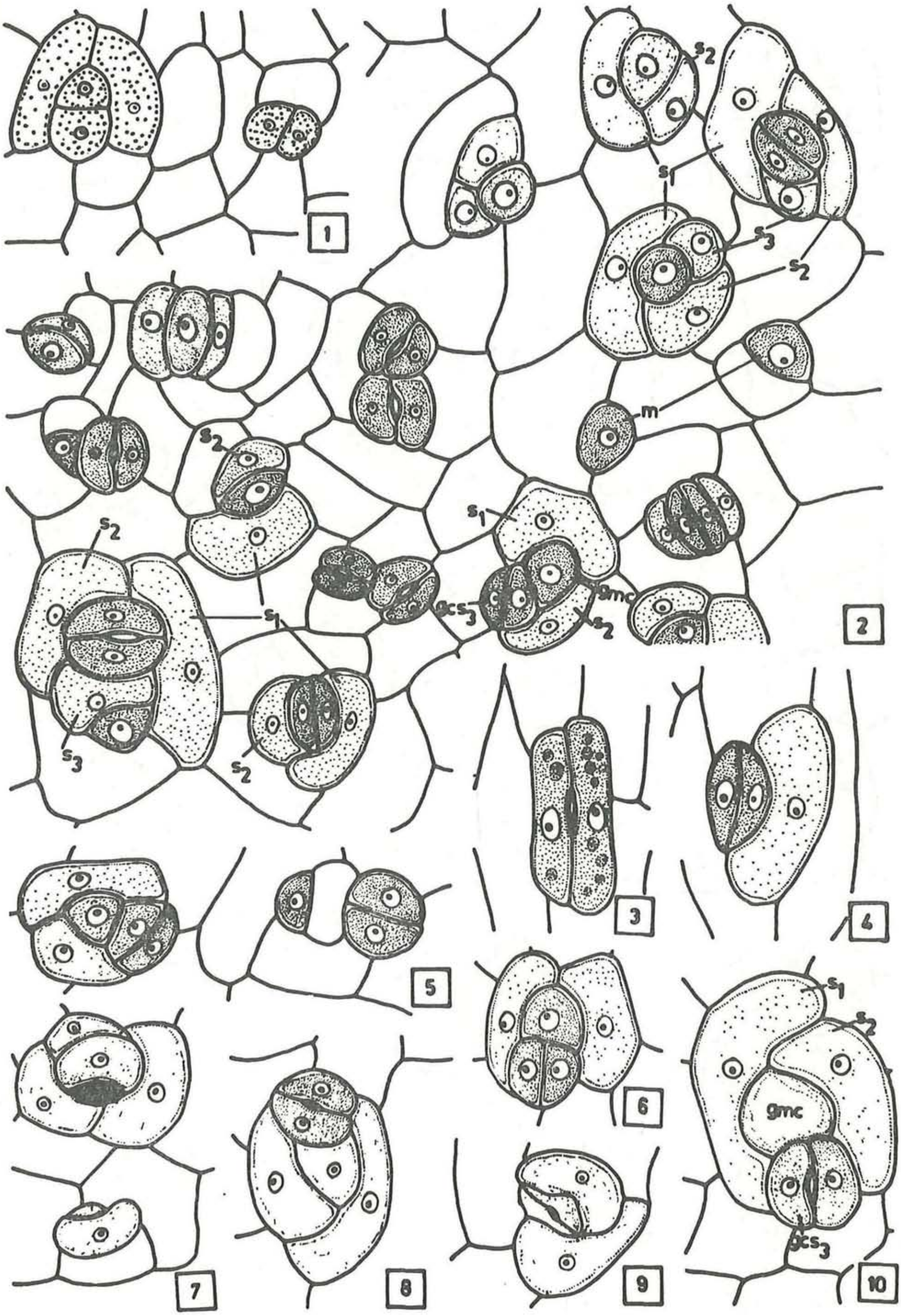
[1]) L = Length, [2]) B = Breadth

Explanation to the text figures:

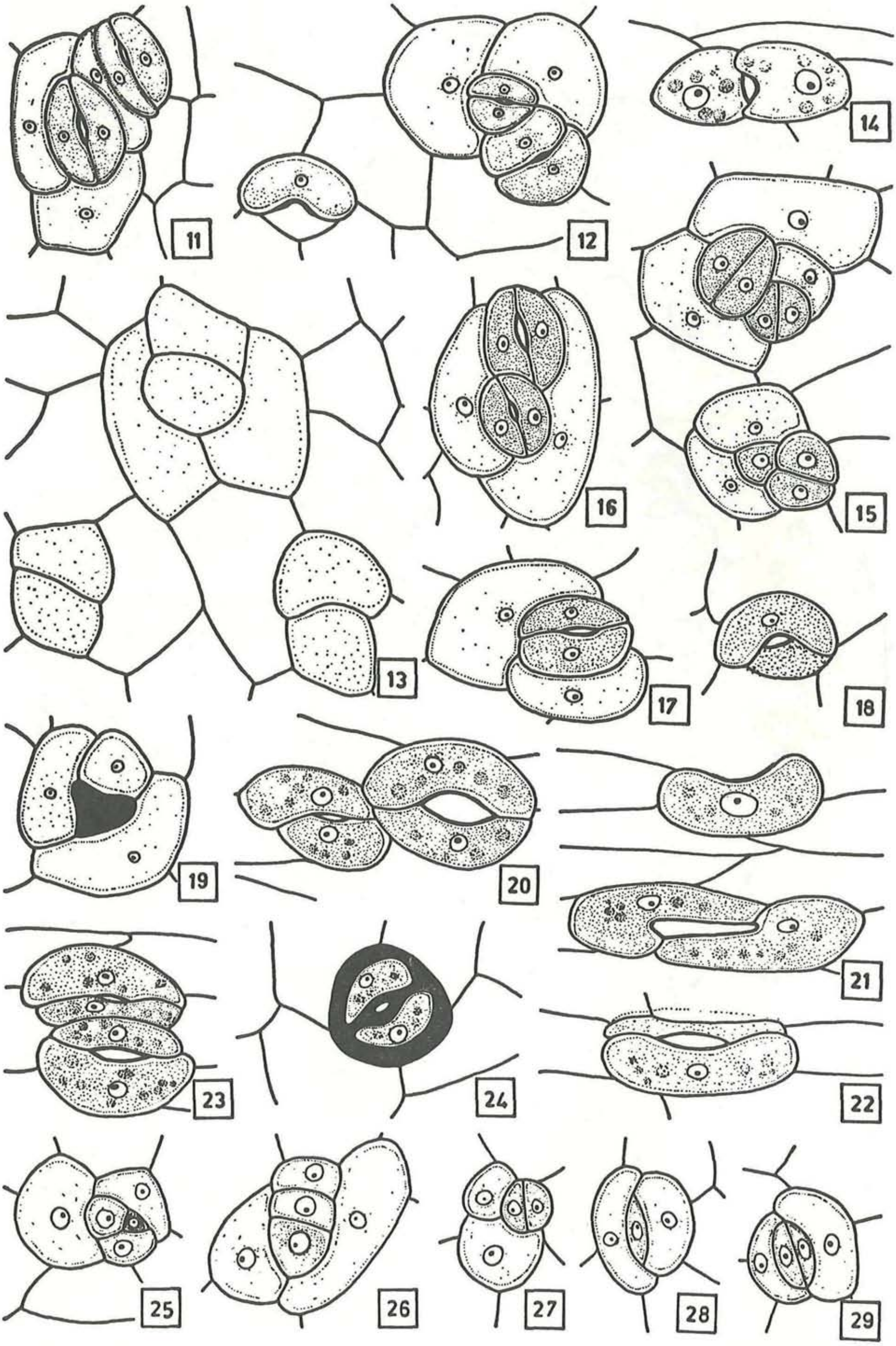
Figs. 1—114. Epidermal peels showing either mature stomata or developmental stages:

Cotyledon: Figs. 1, 2, 5—19, 24, 25—33, 35—41, 43—49, 51—61, 63—74, 76—87, 89, 92—96, 101, 103—111, 113, 114;

Hypocotyl: Figs. 3, 4, 20—23, 34, 42, 50, 62, 75, 88, 90, 91, 97—100, 102, 112 (Abbreviations see the text)



Figs. 1—10. 1: Cleared cotyledon (Cot); 2: Cot, control (DW); 3,4: Hypocotyl (Hyp), control (DW) 5—10: Cot, GA 25 ppm (880×)



Figs. 11—29. 11—14: Cot, GA 25 ppm; 15—19: Cot, GA 50 ppm; 20—23: Hyp, GA 50 ppm; 24: Cot, GA 100 ppm; 25—29: Cot, IAA 25 ppm (880 ×)

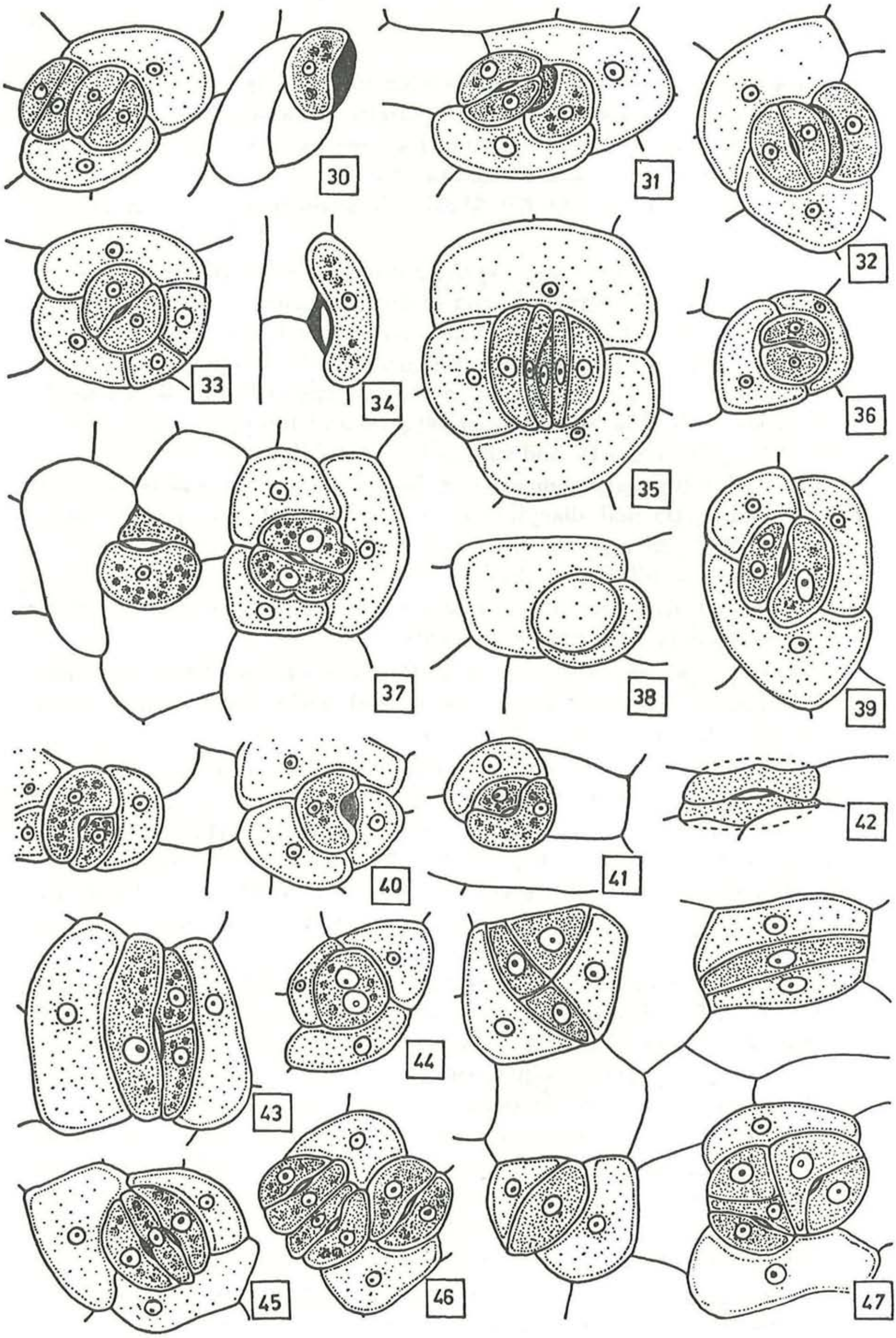
stomata are distributed all over the surface of the cotyledons, rarely over the veins and without any definite pattern of orientation. Sometimes, the nucleus of an epidermal cell undergoes one to many divisions but not followed by subsequent wall formation. So all the nuclei are enclosed in the same epidermal cell (Fig. 66). Multinucleate epidermal cells are noticed in COL treatment.

Mature stomata: Anomocytic, anisocytic, paracytic, hemidiacytic and with a single subsidiary cell are the stomatal types observed in control, GA 25 ppm, 50 ppm, 100 ppm; TIBA 25 ppm, 50 ppm, 100 ppm; SUC 2000 ppm; MH 25 ppm, 50 ppm, 100 ppm; COU 25 ppm, 50 ppm and 100 ppm; anomocytic, anisocytic, diacytic, paracytic and hemidiacytic stomata are noticed in IAA 25 ppm, 50 ppm and 100 ppm; while anomocytic, anisocytic, paracytic and with a single subsidiary cell in COL 25 ppm, 50 ppm and 100 ppm; anomocytic, anisocytic, paracytic and transitional between paracytic and diacytic are noticed in SUL 25 ppm, 50 ppm, 100 ppm; MOR 25 ppm, 50 ppm and 100 ppm. Anisocytic stomata are most common in all the treatments and diacytic are very rarely found in IAA treatment. Paracytic stomata are more frequently found in MOR treatment than in any other treatments.

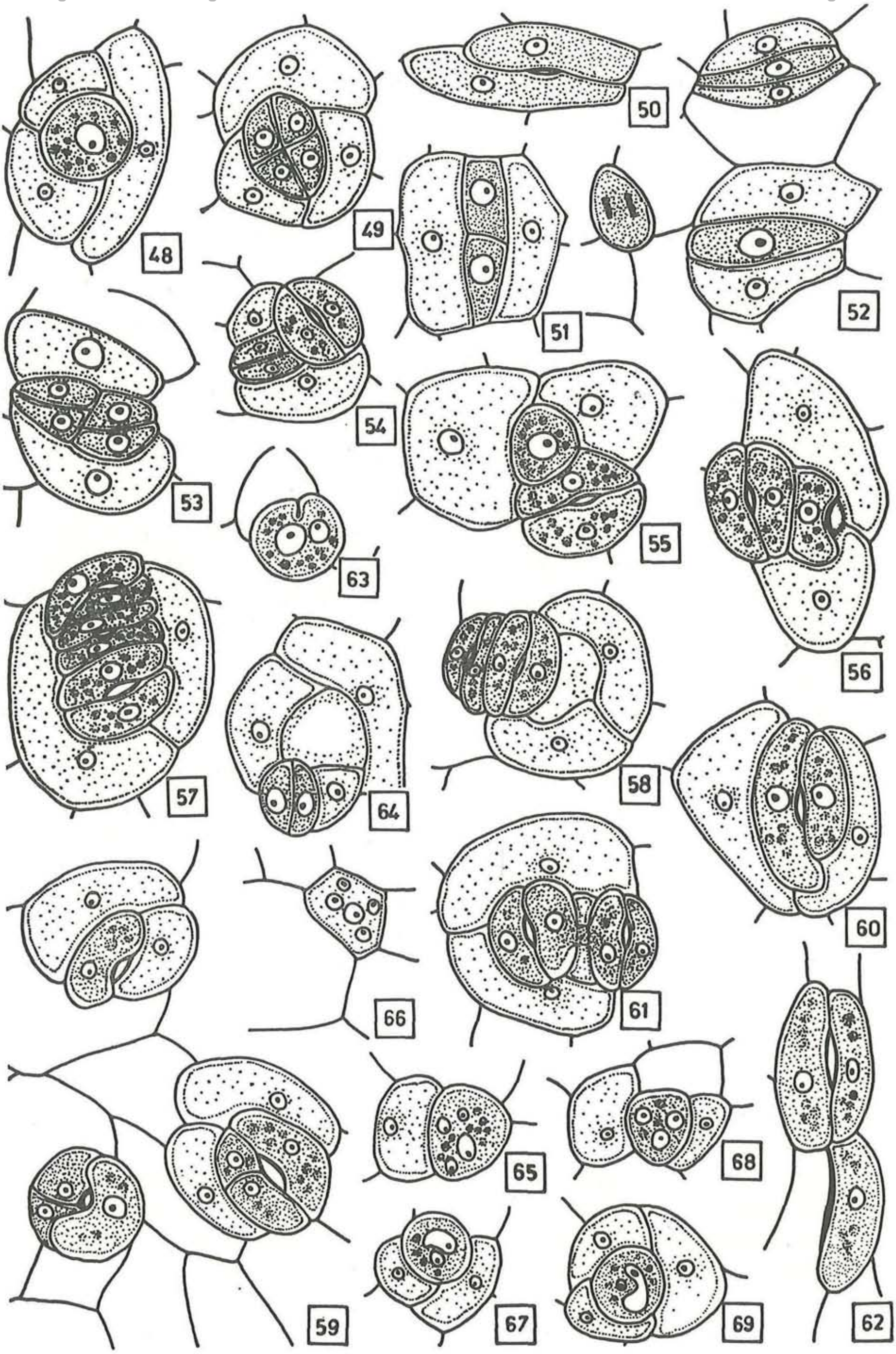
Diverse types of stomata occur on the same surface of the cotyledons while in the hypocotyl mostly anomocytic and rarely stomata with a single subsidiary cell are observed.

Anomocytic stomata are surrounded by 3—6 ordinary epidermal cells (Figs. 2, 3, 18, 110). Paracytic stomata are flanked by 2—3 parallel subsidiary cells either non-contiguous at both the poles (Figs. 43, 110) or contiguous at one (Figs. 17, 60, 105, 110, 114) or both the poles (Figs. 105, 110, 114). In diacytic stomata the two subsidiary cells enclose the guard cell whose cross walls are at right angles to the guard cells (Fig. 36). Anisocytic stomata are surrounded by three to four spirally arranged subsidiary cells of which one is distinctly smaller than the others (Figs. 37, 39, 110, 114). In anisocytic stomata division of the smallest subsidiary cell is a common feature (Figs. 2, 15, 33). Stomata with a single subsidiary cell are flanked by parallel single subsidiaoy cells (Figs. 41, 89). Stomatd which are transitional between paracytic and diacytic are surrounded by two subsidiary cells whose cross walls are obliquely oriented to the guard cells (Fig. 101). In the hemidiacytic stoma the two subsidiary cells are contiguous at one end and non-contiguous at the other end. The cross wall of two subsidiary cells is at right angles to the guard cells (Fig. 27).

Aberrant developments: The following aberrant developments are noticed; (i) 2—3 anomocytic contiguous stomata, (ii) 2—3 anisocytic contiguous stomata, (iii) Twin anisocytic stomata, (iv) Twin paracytic stomata, (v) One and a half contiguous stomata, (vi) Anomocytic single guard cells, (vii) Anisocytic single guard cells, (viii) paracytic single guard



Figs. 30—47. 30—33: Cot, IAA 25 ppm; 34: Hap, IAA 25 ppm; 35—41: Cot, IAA 50 ppm; 42: Hyp, IAA 100 ppm; 43: Cot, TIBA 25 ppm; 44—47: Cot, TIBA 50 ppm; 48: 49, Cot, TIBA 50 ppm (880 ×)



Figs. 48–69. 48, 49: Cot, TIBA 50 ppm; 50: Hyp, TIBA 50 ppm; 51–61: Cot, TIBA 100 ppm; 62: Hyp, TIBA 100 ppm; 63: Cot, TIBA 100 ppm; 64: Cot, SUC 2000 ppm; 65–69: Cot, COL 25 ppm (880 ×)

cells, (ix) Degeneration of guard mother cell, (x) Arrested development, (xi) Degeneration of guard cells, (xii) Thickening of the wall around the guard cells, (xiii) Persistent stomatal cells, (xiv) Division of guard cells, (xv) Obliquely oriented guard cells, (xvi) Cytoplasmic connections between adjacent stomata, (xvii) Stomata without pores, (xviii) Multinucleate guard cells, (xix) Unequal guard cells and (xx) Division of guard mother cell.

Development: The stomatal meristemoids are cut off either in a corner or on one side of protodermal cells (Figs. 2, 5, 52). The meristemoids can be easily distinguished from remaining epidermal cells by their smaller size, prominent nuclei and dense staining properties (Figs. 2, 5, 52). Meristemoids are either triangular, lenticular or spherical in shape and occur solitary or in pairs. The ontogeny of different types of stomata is as follows:

I. Normal stomata:

(a) Anomocytic: The meristemoid directly functions as a guard mother cell without cuting off any subsidiary cells. It enlarges and divides by a straight wall to produce a pair of guard cells between which a lenticular pore develops (Figs. 2, 5). Mature stoma is surrounded by 3—6 ordinary epidermal cells.

(b) Anisocytic: The meristemoid divides by a slightly curved wall to form two unequal cells. The larger cell differentiates as a first subsidiary cell (s_1) while the smaller cell enlarges and divides by a slightly curved wall perpendicular to the first to give rise to two unequal cells. The larger cell differentiates as a second subsidiary cell (s_2). The smaller cell enlarges and divides on its third side by a wall perpendicular to first and second to form an outer third subsidiary cell (s_3) and a central smaller cell. The central cell sometimes cuts off a fourth subsidiary cell in a spiral fashion and then functions as a guard mother cell (Figs. 2, 25, 26). The guard mother cell (gmc) enlarges and divides by a straight wall to produce a pair of guard cells (Fig. 2). Here the meristemoid behaves like an apical cell with three cutting faces and cuts off three to four subsidiary cells in a spiral fashion. If three mesogene subsidiary cells are only cut off then the resulting stomatal type will become anisocytic and if four mesogene subsidiary cells are cut off by the meristemoid, the resulting stomatal type will conform to the helicocytic type of Payne (1970).

(c) Paracytic: Meristemoid divides to form two unequal cells by a curved wall. The larger cell differentiates as a first subsidiary cell (s_1). The smaller cell enlarges and divids parallel to the first on the other side to form two unequal cells. The larger outer cell differentiates as a second subsidiary cell (s_2) and the smaller middle cell functions as a guard mother cell, divides by straight wall parallel to the subsidiaries, to produce a pair of guard cells (Figs. 2, 28, 29). The subsidiary cells may be non-intersecting or intersecting at one or both the poles (Figs. 2, 28, 29, 110, 114).

(d) Diacytic: Here the development is similar to that of paracytic stoma up to the triad stage where the subsidiary cells are intersecting at both the poles. The only difference is that the middle guard mother cell divides at right angles to the subsidiary cells (Figs. 28, 36).

(e) Transitional between paracytic and diacytic: Here also the ontogeny is similar to that of either paracytic or diacytic up to triad stage. The middle guard mother cell divides by an oblique wall so that the cross walls of the subsidiary cells are obliquely oriented to the guard cells (Figs. 28, 101).

(f) Hemidiacytic: The meristemoid divides twice to produce two subsidiary cells which are contiguous at one pole and then functions as a guard mother cell. The guard mother cell divides by a straight wall at right angles to the subsidiary cells to form two guard cells (Figs. 2, 27). Here the subsidiary cells surround the major portion of the guard cells except on one side. The cross wall of the subsidiary cells is at right angles to the guard cells on one side only.

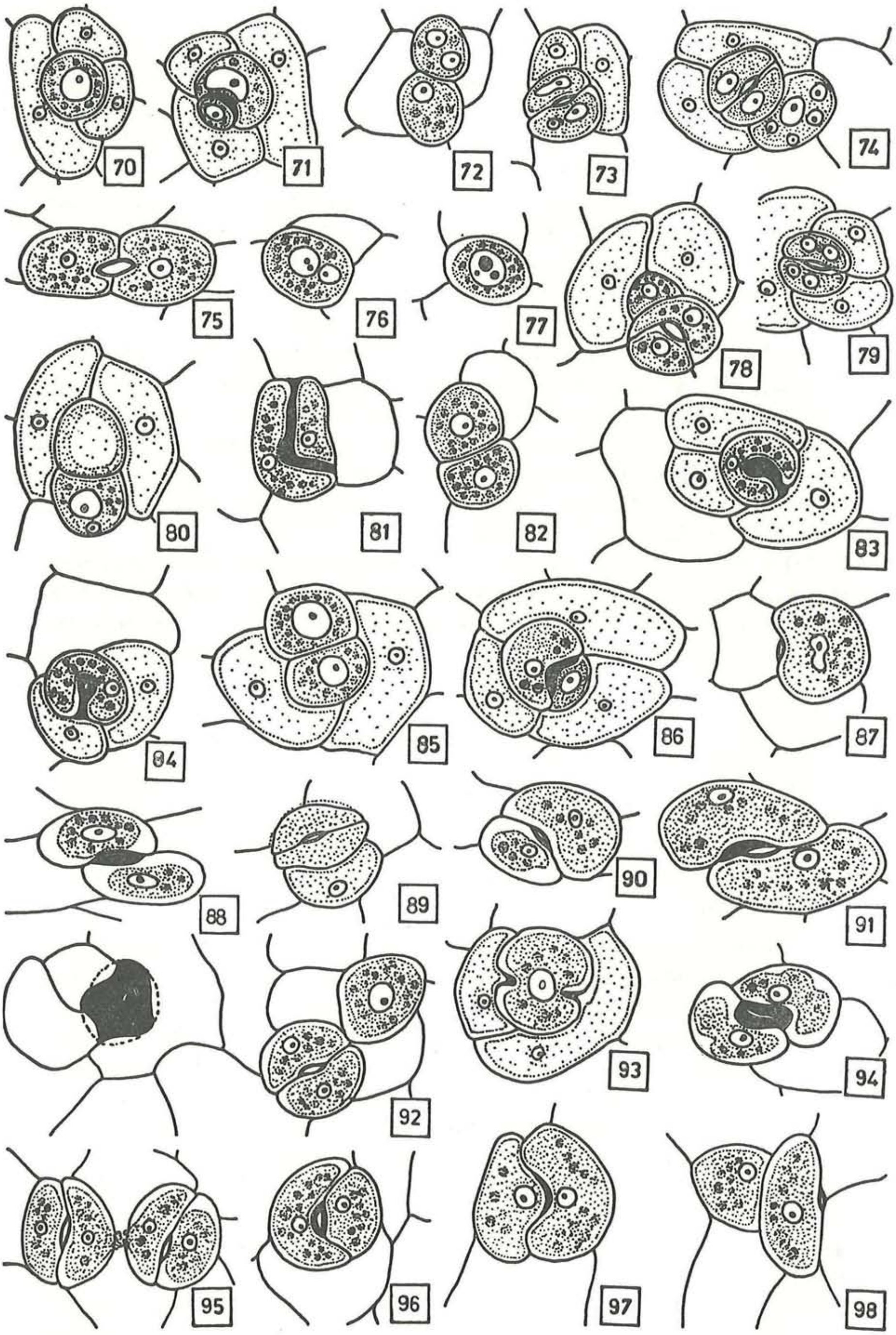
(g) Stoma with a single subsidiary cell: Here the meristemoid cuts off a single subsidiary cell by a slightly curved wall and then functions as a guard mother cell. The guard mother cell divides to produce two guard cells (Fig. 2). The mature stoma is flanked by a single subsidiary cell.

II. Aberrant developments:

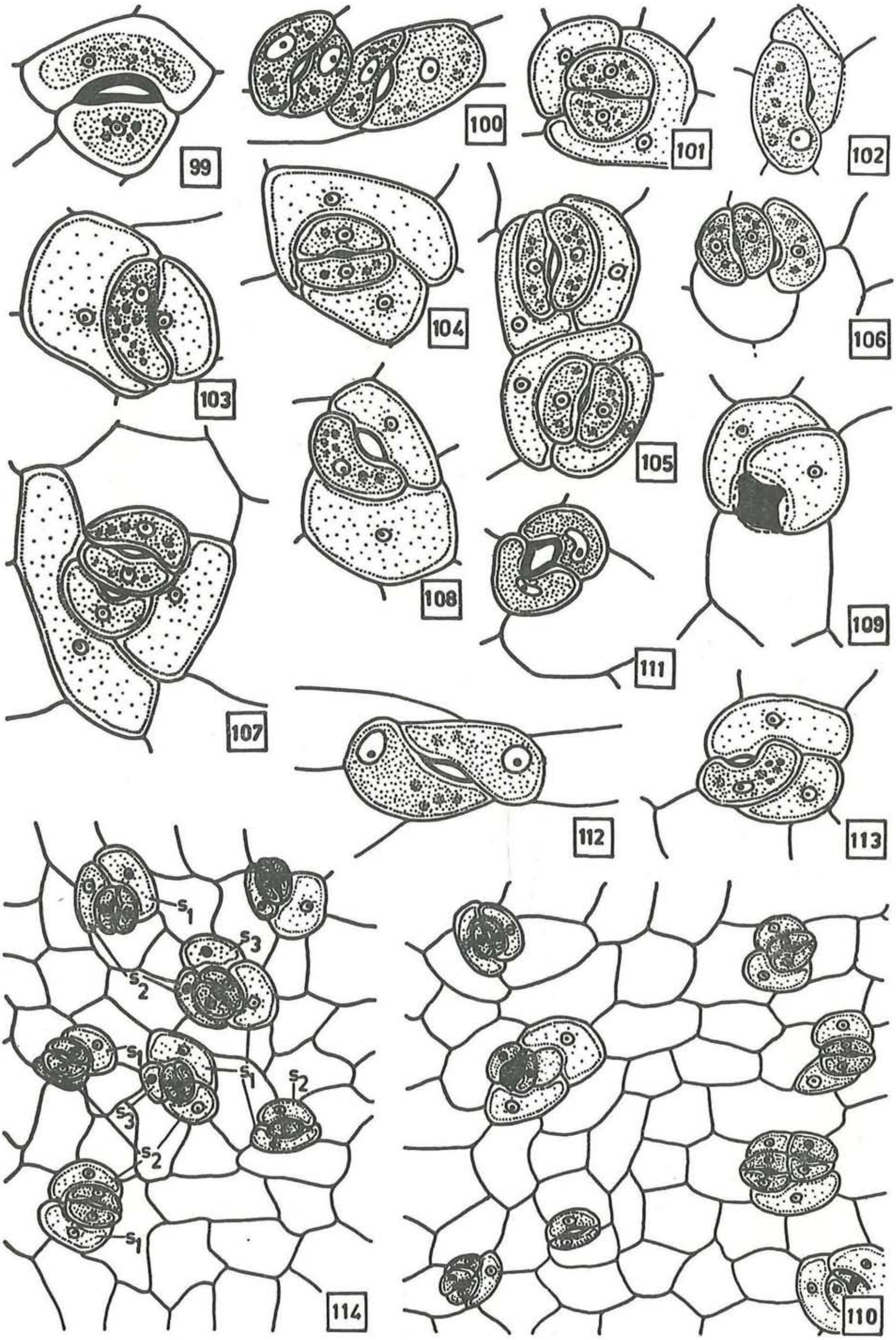
(i) 2—3 anomocytic contiguous stomata: Here the contiguous stomata develop from adjacent meristemoids. Contiguous stomata may be either juxtaposed (Figs. 23, 100) or superimposed (Figs. 2, 20) or at right angles to each other (Fig. 2). These are observed in both untreated and treated seedlings.

(ii) 2—3 anisocytic contiguous stomata: The meristemoid cuts off three subsidiary cells and then functions as a guard mother cell which divides and gives rise to a stoma. The smallest (third) subsidiary cell regains the meristematic activity and divides directly to form two guard cells. This stoma becomes contiguous with a stoma derived from the guard mother cell (Figs. 2, 12, 16, 30, 116). In some cases, the third subsidiary cell divides and its derivative/s develop into stoma/ta which results in 2—3 contiguous stomata (Figs. 2, 15, 57, 58). Contiguous stomata are either juxtaposed or superimposed or at right angles to each other or obliquely oriented depending upon the plane of division in the guard mother cell and derivative/s of the subsidiary cells. These are observed in untreated and treated cotyledons.

(iii) Twin anisocytic stomata: The meristemoid cuts off three subsidiary cells and a central cell which is normally destined to function as a guard mother cell, enlarges and divides again to form two initials.



Figs. 70—98. 70—74: Cot, COL 25 ppm; 75: Hyp, Col 25 ppm; 76—84: Cot, COL 50 ppm; 85: Cot, COL 100 ppm; 86, 87: Cot, MH 25 ppm; 88: Hyp, MH 25 ppm; 89: Cot, MH 50 ppm; 90, 91: Hyp, MH 50 ppm; 92—95: Cot, MH 100 ppm; 96: Cot, COU 25 ppm; 97, 98: COU 100 ppm (880 ×)



Figs. 99–114. 99: Hyp, COU 100 ppm; 100: Hyp, SUL 25 ppm; 101: Cot, SUL 50 ppm; 102: Hyp, MOR 25 ppm; 103–106: Cot, MOR 25 ppm; 107–110: Cot, MOR 100 ppm; 111: Cot. MOR 100 ppm; 112: Hyp, MOR 100 ppm; 113, 114: Cot, MOR 100 ppm; (Figs. 99–109, 111–113 880×, figs. 110, 114 350×)

These two initials derived from the central cell function as guard mother cells, divide and give rise to two contiguos stomata which are then enclosed within three subsidiary cells (Fig. 49). As both the stomata develop from the derivatives of the same initial are described as twin stomata. Twin anisocytic stomata are observed in MH 25 ppm and 50 ppm.

(iv) Twin paracytic stomata: The meristemoid cuts off two parallel subsidiary cells and then instead of functioning as a guard mother cell divides at right angles to the subsidiary cells to produce two initials. These two initials function as guard mother cells and give rise to two stomata which remain contiguous to each other. Here also the two contiguous stomata originate from the derivatives of the same initial, hence called twin stomata (Figs. 51, 53). Rarely, a subsidiary cell cuts off an initial cell which develops into a stoma contiguous with twin stomata (Figs. 46, 47). Twin paracytic stomata are observed in TIBA 50 ppm, 100 ppm and MH 50 ppm.

(v) One and a half contiguous stomata: One and a half contiguous stomata develop in the following ways:

(a) From two adjacently placed meristemoids: Here one of the meristemoids develops into a stoma while the other meristemoid differentiates as a single guard cell (Figs. 62, 106).

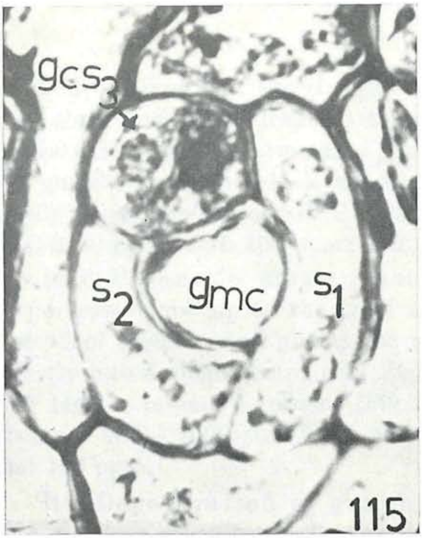
(b) In an anisocytic stomatal development, a third subsidiary cell forms a stoma while the central cell (guard mother cell) differentiates into a single guard cell (Figs. 56, 107). Sometimes, the guard mother cell divides to form two guard cells of which one degenerates before pore formation (Fig. 31). Both these conditions result in one and a half contiguous stomata. Sometimes, one of the guard cells of a stoma derived from the third subsidiary cell degenerates resulting into one and a half contiguous stomata (Fig. 32).

(c) The meristemoid after cutting off 2—3 subsidiary cells divides to produce two initials of which one develops into a normal stoma and the other into a single guard cell (Fig. 45).

One and a half contiguous stomata are observed in IAA 100 ppm, TIBA 50 ppm, 100 ppm; COU 25 ppm, 50 ppm; and MOR 50 ppm.

(vi) Anomocytic single guard cell: Anomocytic single guard cells are formed in the following ways: (a) The meristemoid does not divide at all, it enlarges, gets notched on one side, becomes bean shaped, a differential thickening appears in the region of the notch and a pore may or may not develop (Figs. 12, 21, 34, 87). (b) By the degeneration of one of the guard cells (Figs. 7, 30) of a stoma. These are observed in GA 25 ppm, 50 ppm, 100 ppm; IAA 25 ppm, 50 ppm; TIBA 100 ppm; COL 25 ppm; MH 25 ppm, 100 ppm, COU 25 ppm, 50 ppm, 100 ppm and SUL 100 ppm.

Figs. 115—118: Epidermal peels from the abaxial surface of the cotyledon (2200 ×)



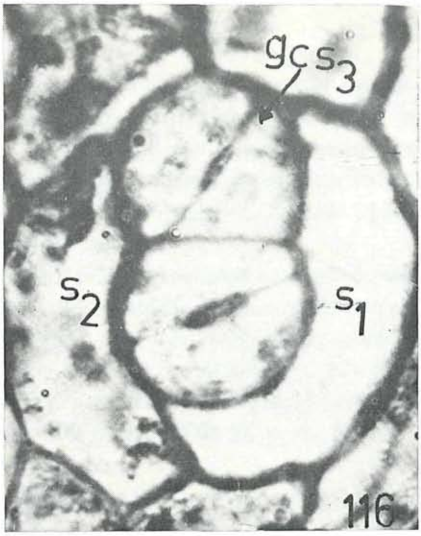


Fig. 115. GA 25 ppm: Note the arrested guard mother cell (gmc) and development of stomata from third subsidiary cell (gcs_3); s_1 = first subsidiary cell, s_2 = second subsidiary cell;
Fig. 116: COU 100 ppm: Note the development of one of the stomata from guard mother cell and the other from third sibsidiary cell (gcs_3)

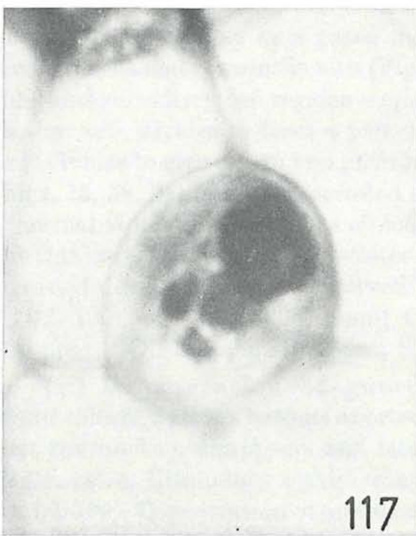


Fig. 117: COL 25 ppm: Multinucleate persistent stomatal cell
Fig. 118: COL 50 ppm: Persistent stomatal cell with large nucleus

(vii) Anisocytic single guard cell: Here the meristemoid undergoes three successive divisions to produce three subsidiary cells and a central cell which functions as a guard mother cell, divides and produces two guard cells out of which one degenerates before pore formation (Figs. 7, 40). These are noticed in GA 25 ppm; IAA 50 ppm; TIBA 25 ppm; SUC 2000 ppm; COL 100 ppm; MH 50 ppm and 100 ppm.

(viii) Paracytic single guard cell: The development conforms to paracytic type up to the triad stage. The middle guard mother cell instead of dividing and giving rise to a pair of guard cells differentiates directly into a single guard cell (Figs. 59, 103). Pore may (Figs. 59, 108, 113, 114) be present or absent (Fig. 103). Paracytic single guard cells are observed in GA 100 ppm; TIBA 50 ppm, 100 ppm; MOR 25 ppm, 50 ppm and 100 ppm.

(ix) Degeneration of guard mother cell: Here the meristemoid cuts off three subsidiary cells and then functions as a guard mother cell. The guard mother cell fails to divide to give rise to two guard cells and degenerates (Fig. 19). The degenerated guard mother cell is seen as a blob in the centre of three subsidiary cells. This is observed in GA 50 ppm.

(x) Arrested development: The stomatal ontogeny may get arrested at any stage of development. It may be at a meristemoid stage or after cutting off 1, 2 or 3 subsidiary cells (Figs. 13, 38). Sometimes a meristemoid cuts off three subsidiary cells and a central cell. The central cell instead of functioning as a guard mother cell, gets arrested in its further development and remains in situ (Figs. 10, 58, 64, 80, 110, 115). Sometimes, the third subsidiary cell regains meristematic activity, functions as a guard mother cell, divides to form a pair of guard cells (Figs. 10 (gcs_3), 15, 115) or it divides to give rise to two initials one or both of which produce stomata (Figs. 15, 58, 64). Here the arrested development becomes contiguous with a normal stoma or contiguous stomata. Rarely in COL 50 ppm treatment the third subsidiary cell differentiates into a persistent stomatal cell (Fig. 80). Arrested developments are observed in GA 25 ppm, 50 ppm; IAA 50 ppm; TIBA 100 ppm; SUC 2000 ppm; COL 50 ppm; MH 25 ppm, 50 ppm, 100 ppm; COU 50 ppm, 100 ppm; SUL 50 ppm and MOR 100 ppm.

(xi) Degeneration of guard cells: Sometimes one or both the guard cells of a stoma become aborted and degenerate. During degeneration first the nucleus disappears and later cytoplasm becomes vacuolated and degenerates. Ultimately a thickening is left around the pore (Figs. 9, 22, 42, 92, 109). Degeneration of guard cells is observed in control, GA 100 ppm; COL 25 ppm; TIBA 25 ppm, 50 ppm, 100 ppm; COU 25 ppm, 100 ppm; SUL 25 ppm; MH 50 ppm, 100 ppm and MOR 50 ppm.

(xii) Thickening of the wall around the guard cells: Very rarely an unusual thickening of the cell wall develops around the guard cells and pore (Fig. 24). This is very rarely observed in GA 100 ppm.

(xiii) Persistent stomatal cells: The meristemoid either (a) directly develops into a persistent stomatal cell or (b) it cuts off 2—3 subsidiary cells and later develops into a persistent stomatal cell or (c) rarely a third subsidiary cell of an anisocytic stoma differentiates into a persistent stomatal cell (Figs. 44, 48, 63, 65, 67—69, 70, 72, 74, 76—78, 80—82, 84, 85, 92, 93, 117, 118). During the development of a persistent stomatal cell, the meristemoid fails to divide before or after cutting off any subsidiary cells. It enlarges, chloroplasts appear, wall becomes uniformly thickened and differential thickening does not develop; differentiates as a persistent stomatal cell. The persistent stomatal cell simulates with the guard cells of a stoma in staining properties. Sometimes, the nucleus of a persistent stomatal cell divides to form two to several nuclei which are enclosed in the same persistent stomatal cell (Figs. 44, 63, 65, 67—69, 70, 72, 74, 76, 77, 117). The persistent stomatal cell varies in size and shape. It may be spherical, oval, triangular, crescentic, or hour-glass shaped (Figs. 44, 48, 55, 63, 65, 67—69, 70, 74, 76, 78, 80—82, 84, 85, 92, 93). Sometimes, there is an ingrowth of the wall of a persistent stomatal cell which may or may not enclose a pore (Figs. 83, 84). Persistent stomatal cells occur either solitary (Figs. 44, 48, 65, 67—69, 70, 76, 83, 84, 93) or in pairs (Figs. 72, 82, 85) or contiguous with arrested development (Fig. 80) or with a single guard cell (Fig. 98). Persistent stomatal cells are very common in COL treatment. They are also observed in TIBA 100 ppm; COU 100 ppm; MH 25 ppm, 50 ppm and 100 ppm.

(xiv) Division of guard cell: One of the guard cells of a stoma undergoes transverse division resulting in equal or unequal but always nucleate cells (Figs. 43, 59). Division of guard cells is noticed in TIBA 50 ppm, 100 ppm and MH 100 ppm.

(xv) Obliquely oriented guard cells: Normally the guard mother cell divides by a straight wall and gives rise to two equal guard cells, but sometimes in treated plants it divides by an oblique division resulting in obliquely oriented guard cells (Figs. 21, 94, 112). Obliquely oriented guard cells are noticed in GA 50 ppm; MH 25 ppm, 50 ppm and MOR 100 ppm.

(xvi) Cytoplasmic connection between adjacent stomata: Sometimes, the opposite guard cells of neighbouring stomata are connected with each other by a cytoplasmic strand (Figs. 61, 95). The cytoplasmic connections bring about symplastic connections between two adjacent stomata and are observed only in the mature epidermis. Therefore, they are secondary in origin, Cytoplasmic connections are observed in TIBA 100 ppm and MH 100 ppm.

(xvii) Stoma without pore: The guard mother cell divides normally to give rise to two guard cells, a thickening appears between the guard cells but an intervening pore does not develop (Figs. 81, 86, 88, 97). These are noticed in COL 25 ppm, 50 ppm; MH 25 ppm and COU 100 ppm.

(xviii) Multinucleate guard cells: The nucleus of one or both the guard cells of a stoma undergoes division to form two nuclei, but the intervening wall formation fails. Hence both the nuclei remain within the same guard cell/s (Figs. 39, 73, 86). Multinucleate guard cells are observed in IAA 50 ppm, COL 25 ppm, 50 ppm.

(xix) Unequal guard cells: The guard mother cell divides unequally to produce two unequal cells instead of producing a pair of guard cells, one of the guard cells may encroach upon the other by rapid enlargement (Figs. 20, 40, 41, 50, 96, 99). Unequal guard cells are noticed in IAA 50 ppm; COL 25 ppm; MH 50 ppm, 100 ppm; COU 25 ppm, 50 ppm and 100 ppm.

(xx) Division of guard mother cell: Very rarely in an anisocytic development of a stoma, the meristemoid after cutting off three subsidiary cells undergoes eihter a ring-shaped division or repeated divisions. In the latter case, the cells thus formed remain as such within three subsidiaries (Figs. 35, 71). This is noticed in IAA 60 ppm and COL 25 ppm.

Discussion

According to METCALFE & CHALK (1950) the stomata are anomocytic in the *Malvaceae*. INAMDAR & CHOHAN (1969a, 1969b) reported anomocytic, paracytic and anisocytic stomata in some members of the *Malvaceae* and *Bombacaceae* and *Hibiscus rosa-sinensis*. In addition to these types, diacytic stomata are reported in *Abelmoschus esculentus* MOENCH by INAMDAR (1970). He also pointed out that in ascorbic acid treatment sometimes the subsidiary cell becomes meristematic and cuts off a meristemoid which begins its own series. INAMDAR (1970), INAMDAR & GANGADHARA (1975) and GANGADHARA & INAMDAR (1975) described the development of persistent stomatal initials. In the present study the stomatal types observed conform to the types described by INAMDAR & CHOHAN (1969a, 1969b) and INAMDAR (1970). In addition to these types stomata with single subsidiary cells, transitional between paracytic and diacytic and hemidiacytic are also reported. A new term ‚hemidiacytic‘ is used which can be conveniently applied to a stoma with two subsidiary cells whose cross wall is at right angles to guard cells on one side and they are non-contiguous on the other side where the stoma is slightly exposed. Stages showing the stomatal ontogeny have been observed for the first time in the dormant cleared cotyledons dissected out from the seed. The ontogeny of anomocytic stomata is perigenous while that of paracytic, anisocytic, diacytic, hemidiacytic, transitional between paracytic and diacytic, and, stoma with a single subsidiary cell is mesogenous. STRASBURGER (1866—67) reported that the subsidiary cells become meristematic and cut off a series of cells. PALIWAL (1967) also reported one or two but not serial divisions of subsidiary cells. In *Brassica juncea* L. INAMDAR *et al.* (1974) described the subsidiary cell cutting off an initial which develops into a stoma. Several interesting observations are made during present study. They are: 2—3 anomocytic contiguous stomata, 2—3 anisocytic contiguous stomata, twin anisocytic stomata, twin paracytic stomata, one and a half contiguous stomata, anomocytic single guard cells, anisocytic single guard cells, paracytic single guard cells, degeneration of guard cells, division of guard cells, persistent stomatal cells, unusual thickening of the wall around the guard cells, obliquely oriented guard cells, cytoplasmic connections between adjacent stomata, stoma without pore, multinucleate guard cells, unequal guard cells. These are observed in various treatments.

During the development of an anisocytic stoma the meristemoid cuts off three subsidiary cells and a central cell. The central cell (guard mother cell) sometimes fails to divide to form two guard cells, but, it degenerates. The third subsidiary cell or its derivative/s develop into stoma/ta which may or may not be contiguous with the stoma developed from the guard mother cell. Division of subsidiary cells is a common feature. In COL treatment persistent stomatal cells with one to several nuclei with 1—3 nucleoli are observed. Notching of persistent stomatal cells is noticed. An alternative term 'Persistent stomatal cell' is suggested for the old term 'Persistent stomatal initial' since it is differentiated. A persistent stomatal cell differs from a meristemoid by the presence of distinct chloroplasts and staining properties as the guard cells and differs from a guard cell by the lack of differential thickening. INAMDAR, BHATT & PATEL (1973) described and differentiated the 'twin stomata' and 'contiguous stomata' in the *Begoniaceae* and *Gesneriaceae*. In the present investigation also contiguous and twin stomata are noticed. In addition to these, anomocytic single guard cells, anisocytic single guard cells and paracytic single guard cells and, one and a half contiguous stomata with various modes of development are also described. Increased concentration of GA causes increase in stomatal frequency and index. In SUC aberrant developments are rare. MH has inhibitory effect on stomatal index. Higher concentration of SUL tend to be having inhibitory effect on stomatal frequency. In MOR treatment transitional types between paracytic and diacytic stomata are commonly observed. COU has inhibitory effect on stomatal index.

According to KLEBS (1903, 1904) the aberrant developments may be due to "Specific nature" while according to BÜNNING (1956) the spatial differentiation of stomata is controlled by the extent and pattern of inhibitory zone around the meristemoid or developing stomata. MORGAN (1934) described them as due to "Cytoplasmic heterogenity" and DEHNEL (1961) to "Intrinsic instability". LALORAYA *et al* (1972) state that "high cytokinin and low auxin seem to be involved in stomatal differentiation, The specificity of differentiation may be related to the type of flavanoid present in the differentiating cells". We believe that the differentiation of a meristemoid into a stomatal complex may be controlled by the specific intrinsic factors at the region of the meristemoid and these factors are disturbed by the exogenous application of growth regulators which ultimately may lead to such aberrant developments. Here, the stomatal anomalies are induced by the exogenous application of growth regulators on the hypocotyl and cotyledons of *Gossypium herbaceum* var. digvijay. The results may be true only for *Gossypium herbaceum* L. var. digvijay.

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