Action of Growth Regulators on the Cotyledonary Stomata of *Cucumis sativus* L.: Structure and Ontogeny

M. GANGADHARA, and J. A. INAMDAR
Department of Botany, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

Abstract. Anomocytic stomata and stomata with single subsidiary cells are commonly observed. Sometimes a stoma appears anisocytic. Double cytoplasmic connections between nearby stomata and division of guard cells with persistent or degenerating nuclei are seen in GA. One or more divisions of guard cells, displaced guard cells and single guard cells with or without pore are noticed in SUC. Formation of single guard cells is a common feature in TIBA. Paracytic stomata, one and a half stomata and persistent stomatal initials are seen in SUL. COUM seems to be not inhibitory in *Cucumis sativus*. In COL stomata with unequal guard cells, unequal stomatal cells with thickening in between but without intervening pore, stoma with double pores, persistent stomatal initials which may be solitary or in groups with varying shapes and with one or two nuclei of different shapes are noticed. The growth regulators affect the frequency of stomata, epidermal cells; stomatal index; size of guard and epidermal cells.

Considerable data have amassed in the past few decades dealing with the structure and ontogeny of stomata in vascular plants in general and angiosperms in particular (PANT 1965, FRYNS-CLAESSENS and VAN COTTHEM 1973). But, only a handful workers have studied the effect of growth substances on stomatal structure and ontogeny (GUYOT 1964, 1970, GUYOT et al. 1968, HUMBERT and GUYOT 1969, INAMDAR 1970). COOPER et al. (1972) studied the effect of the interaction between abscisic acid and kinetin on the stomatal pore of barley. Recently INAMDAR et al. (1974) and INAMDAR and GANGADHARA 1975 studied the effect of various growth regulators in different concentrations and combinations on stomatal structure and ontogeny. The present studies, therefore, have been undertaken to study the effect of growth regulators on cotyledonary stomata of *Cucumis sativus* in order to add more data to our existing knowledge.

Material and Methods

The seeds of *Cucumis sativus* L., of the Cucurbitaceae were brought from the Institute of Agriculture, Anand. The graded seeds were surface sterilized for 10 min in 10 vol. sodium hypochlorite solution, subsequently washed several times in glass-distilled water and germinated in the sterilized Petri dishes, lined with Whatman filter papers using various substrates under

Received August 27, 1974

292
laboratory conditions. The epidermal peels were taken by direct peel method from fresh as well as fixed material (in FAA). Camera lucida drawings were prepared showing the exact size, shape and number of nuclei and chloroplasts in stomatal cells from epidermal peels stained with Delafield’s haematoxylin and mounted in glycerin.

Mean values of 10 observations showing the frequency of epidermal cells, stomata; stomatal index per mm² and size of guard and epidermal cells are compiled in Table 1.

Results

1. Control (Distilled water-DW) (Figs. 1–2, 75): The epidermal cells are isodiametric, polygonal, uninucleate and thin walled in the cleared dormant cotyledons dissected out from the seeds (Fig. 75). The initiation of stomata was observed only after the emergence of the radicle from the seeds.

The stomatal meristemoids are cut off from the epidermal cells in a corner and can be easily distinguished from other epidermal cells by their dense staining properties and prominent nuclei (Fig. 1). The meristemoid may directly function as guard mother cell, divide and give rise to two guard cells without cutting off any subsidiary cells (Fig. 1). Sometimes the meristemoid divides to form a mesogene subsidiary cell and a guard mother cell. The guard mother cell divides to form two equal guard cells (Fig. 2). The mature stomata are anomocytic and with a single subsidiary cell (Fig. 2).

TREATED WITH GROWTH REGULATORS

2. Gibberellic Acid (GA) 25 ppm (Figs. 3–4); 50 ppm (Figs. 5–10, 76, 79) and 100 ppm (Figs. 11–13, 70): In GA 25 ppm juxtaposed contiguous stomata (Fig. 3); cytoplasmic connection between guard and an epidermal cell in hypocotyl are observed. In GA 50 ppm cytoplasmic connection between nearby stomata (Figs. 5, 79); contiguous stomata which may be superimposed (Fig. 8), at right angles and displaced (Fig. 6) are noticed. Sometimes guard cells of a stoma are surrounded by three subsidiary cells, one or two mesogene and rest perigene. Here the stomatal apparatus appears anisocytic (Fig. 7). Division of one or both the guard cells of a stoma is commonly observed (Figs. 9, 76). The nuclei in the divided guard cells may or may not degenerate. In the hypocotyl contiguous stomata (Fig. 10) are noticed. In GA 100 ppm sometimes the wall around the guard cells and/or pore is very much thickened (Figs. 11–12). In the hypocotyl contiguous stomata (Fig. 13) and stoma with oblique pore placed at right angles to the longitudinal axis of the epidermal cells (Fig. 70) are noticed. In all the three concentrations of GA the stomatal index and size of guard and epidermal cells are higher, but the frequency of epidermal cells is lower and stomatal frequency may be equal, higher or less than the control depending on the concentration (Table 1).

3. Sucrose (SUC) 2000 ppm (Figs. 14–23, 72, 77, 78): Here, the cytoplasmic connection between nearby stomata (Figs. 14, 18); single guard cells with pore (Fig. 15) or without pore (Fig. 16); degeneration of one of the guard cells

Abbreviations used: GA — gibberellic acid; SUC — sucrose; TIBA — 2, 3, 5-triiodobenzoic acid; IAA — indole-3-acetic acid; SUL — sulphanilamide; COUM — coumarin; COL — colchicine; DW — distilled water.
Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Treatments</th>
<th>Frequency of epidermal cells</th>
<th>Frequency of stomata</th>
<th>Stomatal index</th>
<th>Size of guard cells</th>
<th>Size of epidermal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DW</td>
<td>3 322</td>
<td>531</td>
<td>14</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>GA 25 ppm</td>
<td>3 205</td>
<td>531</td>
<td>15</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>GA 50 ppm</td>
<td>2 411</td>
<td>579</td>
<td>19</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>GA 100 ppm</td>
<td>2 640</td>
<td>480</td>
<td>15</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>SUC 2000 ppm</td>
<td>3 086</td>
<td>562</td>
<td>15</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>TIBA 25 ppm</td>
<td>1 786</td>
<td>637</td>
<td>12</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>TIBA 50 ppm</td>
<td>2 248</td>
<td>528</td>
<td>17</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>TIBA 100 ppm</td>
<td>2 096</td>
<td>448</td>
<td>18</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>IAA 25 ppm</td>
<td>4 064</td>
<td>515</td>
<td>11</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>IAA 50 ppm</td>
<td>2 086</td>
<td>601</td>
<td>17</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>IAA 100 ppm</td>
<td>2 856</td>
<td>623</td>
<td>18</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>SUL 25 ppm</td>
<td>1 946</td>
<td>464</td>
<td>19</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>SUL 50 ppm</td>
<td>2 144</td>
<td>432</td>
<td>16</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>SUL 100 ppm</td>
<td>2 032</td>
<td>443</td>
<td>16</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>15</td>
<td>COUM 25 ppm</td>
<td>2 330</td>
<td>574</td>
<td>20</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>COUM 50 ppm</td>
<td>2 653</td>
<td>706</td>
<td>19</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>17</td>
<td>COUM 100 ppm</td>
<td>3 376</td>
<td>523</td>
<td>13</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>18</td>
<td>COL 25 ppm</td>
<td>2 989</td>
<td>222</td>
<td>7</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>COL 50 ppm</td>
<td>2 099</td>
<td>14</td>
<td>0.66</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>COL 100 ppm</td>
<td>1 933</td>
<td>8</td>
<td>0.5</td>
<td>25</td>
<td>15</td>
</tr>
</tbody>
</table>

*L* — length, *B* — breadth.

(Fig. 15) one or more divisions of both the guard cells (Figs. 16, 17, 77) are observed. Sometimes a stoma is associated with 2—3 meristemoids which may or may not give rise to stomata (Fig. 72). In the hypocotyl single guard

---

Epidermal peels showing either developing or mature stomata in the cotyledons (lower) or hypocotyl of *Cucumis sativus*:

Cotyledon: Figs.: 1—3, 5—9, 11, 12, 14—18, 24—29, 31—69, 72, 75.

Hypocotyl: Figs.: 4, 10, 13, 19—23, 30, 70, 71, 73, 74.

Fig. 1. Developmental stages.
Fig. 2. Anomocytic stomata and stoma with single subsidiary cell.
Figs. 3, 6, 10, 13, 19, 29, 30, 36, 42, 45, 47, 69, 74. Contiguous stomata.
Fig. 4. Cytoplasmic connection between a guard cell and an epidermal cell.
Figs. 5, 14, 35. Cytoplasmic connection between nearby stomata.
Fig. 7. Anisocytic stoma.
Fig. 9. Division of guard cells, note guard cells without nuclei.
Figs. 11, 12. Note cuticular thickening around the pore and guard cells in Fig. 11 and only around pore in Fig. 12.
Fig. 13. Single guard cell with pore and degeneration of one of the guard cells.
Fig. 16. Single guard cell and division of guard cells.
Figs. 17, 22. Division of guard cells.
Fig. 18. Contiguous stomata, note the cytoplasmic connection between adjoining guard cells.
Figs. 20, 21. Obliquely placed guard cells with or without pore.
Fig. 23. Single guard cells with and without pore and degeneration of guard cell.
cell with or without pore (Fig. 23); contiguous stomata (Fig. 19); displaced guard cells (Figs. 20, 21) and division of guard cells (Figs. 22, 78) are noticed. The frequency of stomata, stomatal index, size of guard and epidermal cells are higher, but the frequency of epidermal cells is less than control (Table 1).

4. 2,3,5-Triiodobenzoic Acid (TIBA) 25 ppm (Fig. 24); 50 ppm and 100 ppm (Figs. 25—30): The formation of single guard cells is a common feature in all three concentrations (Fig. 24). In TIBA 25 ppm juxtapposed contiguous stomata (Fig. 24); in TIBA 100 ppm superimposed contiguous stomata (Fig. 29); unequal and displaced guard cells (Figs. 25, 26, 27); degeneration of guard cell (Fig. 28) and in hypocotyl contiguous stomata (Fig. 30) are observed. As the concentration of TIBA increases, the stomatal index also increases but the stomatal frequency decreases (Table 1).

Development of Single Guard Cells: The meristemoid does not divide, gets notched on one side and chloroplasts appear. The differential wall thickening appears on the side of the notch. The meristemoid assumes kidney-shape and gets differentiated into a single guard cell without pore (Fig. 24).

5. Indole-3-acetic Acid (IAA) 25 ppm (Figs. 31—32, 74); 50 ppm (Figs. 33—35) and 100 ppm (Fig. 36): In IAA 25 ppm the wall around the guard cells and the adjacent epidermal cells are often greatly thickened (Fig. 31). Meristemoid contiguous with a stoma (Fig. 32) is also noticed. In the hypocotyl contiguous stomata (Fig. 74) are observed. In IAA 50 ppm a meristemoid destined to become a persistent stomatal initial contiguous with a stoma (Fig. 33): cytoplasmic connection between nearby stomata (Fig. 35) and rarely persistent stomatal initial are noticed (Fig. 34).

In IAA 100 ppm juxtapposed contiguous stomata (Fig. 36) are seen. Contiguous stomata are developed from two adjacent meristemoids. As the concentration of IAA is increased, the stomatal index and frequency of stoma is increased while the size of guard cells remains constant (Table 1). In IAA 25 ppm the frequency of epidermal cells is more than control, while in IAA 50 ppm and 100 ppm the frequency and index of stomata are greater but the frequency of epidermal cells is less than control (Table 1).
6. Sulphanilamide (SUL) 25 ppm (Figs. 37–40); 50 ppm (Figs. 41–44, 71) and 100 ppm (Figs. 45–46, 69): In SUL 25 ppm one and a half stomata (Fig. 37), degeneration of guard cell (Fig. 37), persistent stomatal initial which may be solitary or contiguous with each other (Fig. 38), contiguous juxtapposed unequal stomata (Fig. 39) are observed. Here the mature stomata may be anomocytic (Fig. 38), paracytic (Fig. 40) and with a single subsidiary cell (Fig. 40). In SUL 50 ppm a guard cell cutting off a meristemoid-like cell (Fig. 41), single guard cell with pore (Fig. 41), contiguous stomata which may be superimposed (Fig. 42) or juxtapposed (Fig. 44), persistent stomatal initial (Fig. 44) and paracytic stoma with perigenous subsidiary cells (Fig. 43) are noticed. In SUL 100 ppm contiguous stomata which are juxtapposed (Fig. 45) or at right angles (Fig. 69), rarely persistent stomatal initial and single guard cell (Fig. 46) are seen. In all the three concentrations of SUL the frequency of epidermal cells is less and stomatal index is greater than control. As the concentration of SUL increases, the stomatal index and frequency decrease (Table 1).

7. Coumarin (COUM) 25 ppm (Fig. 47); 50 ppm and 100 ppm (Figs. 48-49): In all the three concentrations of COUM single guard cells are very common. In COUM 25 ppm contiguous stomata which are at right angles and displaced (Fig. 47) are observed. In COUM 100 ppm in addition to above, stoma with pore at right angles to the longitudinal axis of guard cells is noticed. As the concentration of COUM increases, the frequency of epidermal cells also increases but the stomatal index decreases (Table 1), and the size of guard and epidermal cells remains constant (Table 1).

8. Colchicine (COL) 25 ppm (Figs. 50–62, 67–68, 73, 80–84); 50 ppm (Fig. 63) and 100 ppm (Figs. 64–66): The increased concentration of COL inhibits the formation of stomata and promotes the induction of persistent stomatal initials and several stomatal anomalies (Figs. 50–68). The aberrant stomatal types seen are: (i) Stomata with unequal guard cells which may result due to unequal division of the meristemoids (Figs. 50, 55, 58). These formations are very common in COL 25 ppm. (ii) Unequal stomatal cells with thickening in between but without intervening pore (Figs. 53, 54, 67, 68). This is due to a ring-shaped division in the stomatal meristemoid (Figs. 67, 68, 83). (iii) Stoma with double pores is observed only in COL 50 ppm (Fig. 62). (iv) Stoma with abnormally big guard cells (Fig. 64). (v) Persistent stomatal initial: The persistent stomatal initials are observed in all the concentrations of COL. In COL 100 ppm the formation of persistent stomatal

---

**Fig. 49.** Pore at right angles to the longitudinal axis of guard cells.

**Figs. 50, 55, 58.** Unequal guard cells.

**Figs. 51, 57.** 2–3 persistent stomatal initials.

**Figs. 52, 53, 54, 67, 68.** Note the unequal stomatal cells with thickening inbetween and without pore.

**Fig. 62.** Stoma with double pores.

**Fig. 64.** Abnormally big guard cells.

**Figs. 70, 71.** Anomocytic stoma.

**Fig. 72.** Stoma associated with a group of meristemoids.

**Fig. 73.** Anomocytic stoma.

**Fig. 75.** Cleared epidermis of dormant cotyledons.
Figs.: 1—9, 11—21, 24—73, 75 × 700; Figs.: 10, 22, 23, 74 × 520.

Cotyledon: Figs.: 76, 77, 79—84.

Hypocotyl: Fig.: 78.

Figs.: 76, 78, 79 × 1360; 77, 80—84 × 2000.
initials is maximum so that the stomatal index per mm$^2$ is insignificant (i.e. 0.5). The stomatal meristemoid sometimes fails to divide, increases in size, chloroplasts appear and differential thickening does not develop and differentiates into a persistent stomatal initial. Sometimes, the nucleus of the meristemoid divides into two but this is not followed by wall formation so that both nuclei remain within the same differentiating persistent stomatal initial (Figs. 59, 60, 81). The two nuclei may be equal or unequal in size (Figs. 59, 60, 81). The persistent stomatal initials may be solitary (Figs. 56, 59--61, 63, 65, 66, 80) or in groups of 2--3 (Figs. 51, 57, 82, 84). The nucleus of the persistent stomatal initial may be centrally or eccentrically located and shows variation in size and shape (Figs. 51, 59--61, 63, 65, 66, 80). The shape of the nucleus may be spherical, crescentric, amoeboid, oval or reniform with 1--2 nucleoli. The nucleoli may be spherical, or erratic in outline. The size of nucleus varies from 3.2 μm to 7.9 μm. As the increase in the size of the nucleus, number of nuclei and nucleoli is an index of chromosomal duplication, such abnormalities induced by colchicine may be showing polyploidy. The persistent stomatal initial may contain few or more chloroplasts. In hypocotyl anomocytic stomata (Fig. 73) are observed.

Discussion

SHARMA and DUNN (1968, 1969) studied the environmental effects on the cuticular and epidermal features of Kalanchoe and Datura respectively. TAL and IMBER (1971) reported that in the leaves of tomato treated with 2,4-Dichlorophenoxyacetic acid, the stomatal density did not increase, the stomata were able to open and close and their aperture width under light was not greater than in untreated leaves. Here, an attempt has been made to study the action of growth regulators on stomatal structure and ontogeny in the cotyledons of Cucumis sativus. RAYYA and RAO (1968) reported that the foliar stomata of Cucumis pubescens are mostly anomocytic being surrounded by 3--5 cells nearly of the same size and similar to the adjacent epidermal cells, however, some of them, tend to be anisocytic. In the present study we have observed stomata which are predominantly anomocytic and sometimes with a single subsidiary cell. In some cases, stoma surrounded by three subsidiary cells look-like anisocytic. In SUL treatment paracytic stomata are noticed as reported by INAMDAR and GANGADHARA 1975 in Lagenaria leucantha. The stomatal development is either mesogenous, mesoperigenous or perigenous. INAMDAR (1970) studied the action of growth regulators on the

---

Fig. 76. Gibberellic acid 50 ppm: Division of both guard cells, note the degeneration of nuclei.
Fig. 77. Sucrose 2000 ppm: Division of guard cells, note the degeneration of nuclei.
Fig. 78. Sucrose 2000 ppm: Division of one of the guard cells.
Fig. 79. Gibberellic acid 50 ppm: Note double cytoplasmic connections between nearby stomata (marked by arrow).
Fig. 80. Colchicine 25 ppm: Persistent stomatal initial with large nucleus.
Fig. 81. Colchicine 25 ppm: Persistent stomatal initial with two nuclei.
Fig. 82. Colchicine 25 ppm: Contiguous persistent stomatal initials.
Fig. 83. Colchicine 25 ppm: Note unequal stomatal cells with two nuclei in larger stomatal cell.
Fig. 84. Colchicine 25 ppm: Group of persistent stomatal initials.
development of stomata of *Abelmoschus esculentus*, and reported the prolonged period of meristematic activity in GA, formation of persistent stomatal initials with equal or unequal division by furrowing in COL; and in COUM maximum inhibition of growth so that even cotyledons failed to emerge out. INAMDAR and GANGADHARA 1975 also noticed the maximum inhibition in COUM where the cotyledons failed to emerge out of the seed coat and formation of persistent stomatal initials and stoma with double pores in COL treatment. We have noticed the induced division of guard cells and the nuclei which may or may not degenerate in the divided guard cells in GA and SUC. GUYOT et al. (1969) also reported the formation of persistent stomatal initials in *Dianthus caryophyllus* by the action of colchicine. GUYOT (1970) states that “treatments of colchicine on plant epidermis make it possible to observe the phenomena of ‘tropocinesis’. Colchicine maintains in the guard mother cell the gradient which was responsible for unequal divisions in the meristemoid initial cell. This gradient may be enhanced by the treatment and the initial cell undergoes a ring shaped division as in the normal floating stomata”. He also explains various instances of polyploid giant stomata. Here we have observed the formation of stomata with double pores, unequal and ring shaped division of the stomatal meristemoid, failure of pore formation and the development of persistent stomatal initials which vary in shape, size with varying size, and number of nuclei and nucleoli. The increase in the size and number of nuclei and nucleoli is an index of chromosome duplication. Growth regulators used in the present study affect the stomatal frequency, stomatal index, frequency of epidermal cells and size of the guard and epidermal cells unlike the report of TAL and IMBER (1971). The increased concentration of COL decreases the stomatal formation and stomatal index and increases the development of persistent stomatal initials. COUM seems to be not inhibitory in *Cucumis sativus*. These results may be true only for *Cucumis sativus* L.

Acknowledgements

M. Gangadhara thanks the University Grants Commission for the award of a research scholarship.

References


BOOK REVIEW


This small book has appeared as a further volume of the series of student texts “Contemporary Biology” edited by E. J. W. Barlington and A. J. Willis. The author, Head of the Botany Department, Rothamsted Experimental Station, and formerly Professor of botany and plant physiology at the Imperial College, University of London, acquaints the reader with the existing knowledge of the mechanism of photosynthesis, as obtained from research in such related sciences as biochemistry and physical chemistry.

The text is divided into 8 chapters: Physiology of photosynthesis; Carbon metabolism; Photorespiration of the chloroplast; Excitation and fluorescence; The physiological evidence for two photochemical reactions in green plants; The comparative biochemistry of photosynthesis; Electron transport in photosynthesis. The first chapter summarizes the fundamental knowledge of photosynthesis in the wider sense, i.e. from the carbon dioxide transport to photo- and biochemical processes. The other chapters are devoted to special problems, such as carbon metabolism of photosynthesis, the biochemical differences between C3 and C4 plants, photorespiration, absorption and emission of radiant energy by plant pigments, mechanism of the process of photosynthesis, etc. The text is supplemented with a list of 149 references, a brief subject index and an appendix where some essential terms are explained.

Although the aim of the book was to provide a “perspective for the university undergraduate and the young research worker” it may surely be useful as a reliable source of digested current information in any laboratory working in plant science.

J. Čatský (Praha)