MORPHOGENETIC EFFECT OF VARIOUS GROWTH REGULATORS ON THE FOLIAR STOMATA OF *VIGNA SINENSIS**

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ABSTRACT

Normal foliar stomata of *Vigna sinensis* (Torner) Savi are of paracytic, anisocytic, anomocytic, cyclocytic and transitional types, the most frequent type being paracytic. Effect of kinetin, Comp. II and 2, 4-D on the stomatal development was studied. Higher concentration of these growth regulators inhibited stomatal development. Following 2, 4-D treatment, cyclocytic stomata are more frequent. With IAA and Comp. II treatments, walls of guard cells become more thickened. Abnormalities, like stomata with one guard cell, unequal guard cells and complete degeneration of guard cells have been observed due to the effect of various growth regulators. The growth regulators also affect the frequency of stomata, stomatal index and the size of epidermal and guard cells.

INTRODUCTION

Considerable information regarding structure and development of stomata in angiosperms has been obtained in the past few decades (PANT, 1965; FRYNS-CLAESSENS & VAN COTTHEM, 1973). However, only a few workers have studied the effect of growth regulators on stomatal structure and ontogeny (GUYOT, 1964; GUYOT *et al.*, 1968; INAMDAR 1970; INAMDAR *et al.*, 1974; SUBRAHMANYAM *et al.*, 1974, etc.). Recently, GANGADHAR AND INAMDAR (1975) and INAMDAR AND GANGADHAR (1975) studied the effect of different concentrations of growth regulators on stomatal structure and ontogeny. The present study has been carried out to understand the effect of various growth regulators on the frequency, morphology and ontogeny of stomata on the first leaflet of *Vigna sinensis* (Torner) Savi.

MATERIAL AND METHOD

The seeds of *Vigna sinensis* for the present study were obtained from the local unit of National Seed Corporation of India. The seeds were surface-sterilized in 10% sodium hypochlorite solution for 10 min., subsequently washed many times in distilled water and germinated in different concentrations of growth regulators in sterilised petri-dishes, lined with Whatman filter paper number 1. Seeds grown in distilled water served as control. The solutions were changed every 24 hours. The first leaflets from the germinated seeds were collected on the eighth day, washed thoroughly and fixed in FAA. Epidermal peels were taken by the direct peel method from the middle region of the leaflets. The epidermal peels were stained in 1% aqueous solution of safranin or Delafieds hematoxylin and mounted in glycerin-jelly. Camera lucida drawings were prepared showing exact size, shape and number of stomata and epidermal cells. The microphotographs have also been taken. The terminology used here is same as suggested by FRYNS-CLAESSENS AND VAN COTTHEM (1973) and DILCHER (1974).

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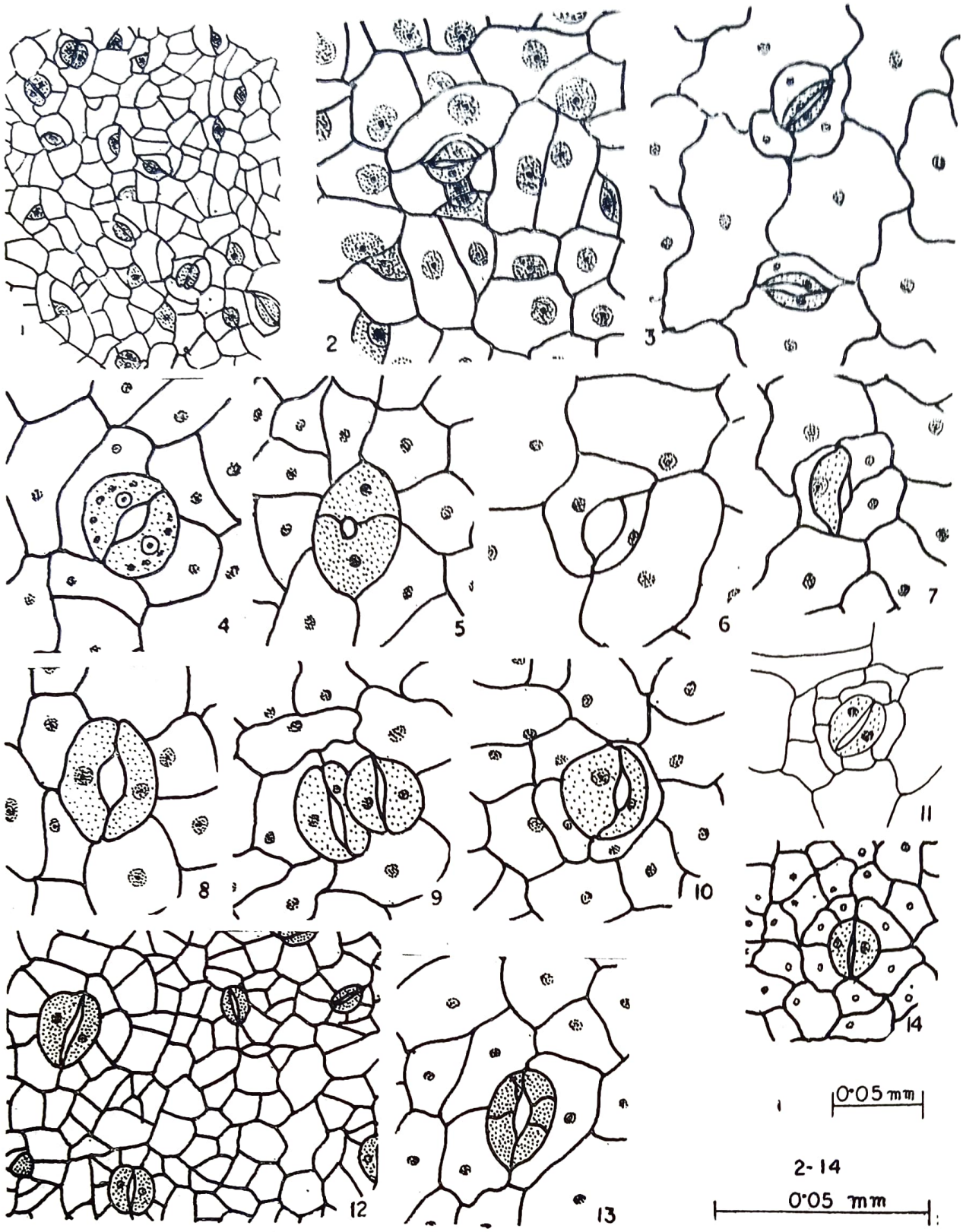
OBSERVATION

The mean value of ten observations showing stomatal frequency per mm², stomatal index and size of guard cells and epidermal cells on abaxial surfaces are compiled in Table 1.

Table 1—Effect of various growth regulators on stomatal frequency per mm², stomatal index and the size of guard cells and epidermal cells on abaxial surface

Treatment		Stomatal frequency per mm²	Stomatal index	Size in μ			
				Guard cells		Epidermal cells	
				Length	Breadth	Length	Breadth
DW		715	17.2	17.5	7.4	28	14
GA	10 ppm	650	11.8	14.2	7.7	24.6	8.4
	50 ppm	653	13.9	12.4	5.6	28.2	12.4
	100 ppm	586	10.1	12.4	5.6	22.4	9.8
2, 4-D	1 ppm	654	21	16.8	5.6	18	7
IAA	10 ppm	388	16.8	16	7	33.6	21
	50 ppm	397	18.6	16	7	36.4	19.6
	100 ppm	456	20.2	15.4	7	34.4	19.6
COUM	10 ppm	782	35.3	19.6	7	25.2	14
	50 ppm	651	33.3	16.8	7	28	16.8
	100 ppm	521	24.2	19.6	7	25.2	14
COL	10 ppm	654	28.6	16.8	8	33.6	19.6
	25 ppm	652	25	15.4	5.6	30.8	16.8
	50 ppm	0	0	0	0	36.4	19.6
Kinetin	1 ppm	516	13.8	16.8	4.2	23.2	16.8
	5 ppm	782	20	16.8	4.2	20.8	14.2
	10 ppm	519	13.7	16.8	4.2	22.4	16
Comp-II	10 ppm	653	25	14	5.6	23.8	11.2
	25 ppm	1304	44.4	12.2	4.2	16.2	8.2
	50 ppm	520	21	14	5.6	22.4	11
CEPA	10 ppm	1434	26.8	9.8	3.5	14	8.5
	50 ppm	912	18.9	11.7	5	16.5	8.8
	100 ppm	847	17.7	14	5	18	9.4

Control (Distilled water=DW)—The epidermal cells are isodiametric or polygonal with thin walls. Stomata are evenly distributed without any definite pattern of orientation (Text-fig. 1).



1. Showing distribution of stomata, the paracytic stomata are most frequent.
2. Stoma contiguous with meristemoid.
3. Epidermal cells showing slightly wavy walls.
4. Stoma showing enough stretched guard cells in width.
5. Transversely divided guard cell mother cell leaving small opening in the centre.
6. Stoma without guard cells.
7. Stoma showing single guard cell.
8. Anomocytic stoma.
9. Contiguous stomata.
10. Stoma showing unequal guard cells.
11. Showing cycloperigenous development of cyclocytic stomata.
12. Frequent occurrence of cyclocytic stomata.
13. Transversely divided guard cells.
14. Cyclocytic stomata

The mature stomata may be paracytic (Pl. 1, Fig. 1), anisocytic (Pl. 1, Fig. 2), anomocytic (Text-fig. 8), cyclocytic (Text-fig. 14) and transitional form (Pl. 1, Fig. 3). Different types of stomata occur on the same surface but paracytic type of stomata are most frequent (Text-fig. 1).

The stomatal meristemoids are usually triangular and can be easily distinguished from other epidermal cells by their dense staining properties. The development of paracytic stomata may be paramesogenous (Pl. 1, Fig. 4) or paramesoperigenous (Pl. 1 Fig. 5). Anisocytic stomata may develop anisomesogenous (Pl. 1, Fig. 6) or anisomesoperigenous (Pl. 2, Fig. 7). Transitional types of stomata may be mesogenous or mesoperigenous in origin. The anomocytic stomata develop aperigenously (Pl. 2, Fig. 8) and cyclocytic stomata develop cycloperigenously (Text-fig. 11).

Very rarely a few abnormalities like contiguous stomata (Text-fig. 9), stoma with a single guard cell (Text-fig. 7), transversly divided guard cells (Text-fig. 13), transversely divided guard mother cell leaving small opening in centre (Text-fig. 5) and unequal guard cells (Text-fig. 10) have been observed.

TREATED WITH GROWTH REGULATORS

1. Gibberelic acid (GA)—With different concentrations of GA, the stomatal index on the whole decreased and both the epidermal cells and the guard cells become smaller (Table 1). The walls of epidermal cells become slightly thickened and the nuclei also become enlarged (Pl. 2 Fig. 9). Often, meristemoids contiguous with stomata (Text-fig. 2) are also noticed.

2. 2,4-dichlorophenoxyacetic acid (2,4-D)—In 1 ppm 2,4-D the stomatal index is higher than in the control and the epidermal cells and guard cells become smaller (Table 1). Further epidermal cells become distinctly polygonal and thick-walled. Also a large number of meristemoids, which failed to develop into stomata, are noticed. An interesting feature is the frequent occurrence of the cyclocytic type of stomata (Text-fig. 12). In higher concentration of 2,4-D (5ppm and 10 ppm) the seeds failed to germinate.

3. Indole-3 acetic acid (IAA)—The stomatal frequency was less in all the three treatments than that in the control, while there was a slight increase in the stomatal index (Table 1). Further the epidermal cells become enlarged (Table 1) and cell walls become slightly wavy (Text-fig. 3). Abnormalities like stomata with single guard cell and stomata without guard cells (Text-fig. 6) are also common.

4. Coumarin (COUM)—With higher concentrations of COUM (50 ppm and 100 ppm) the stomatal frequency decreased while the stomatal index is greater with all the three treatments of COUM to that of the control (Table 1). Also the contiguous stomata and stomata with single guard cell are frequent.

5. Colchicine (COL)—With low concentration of COL (10 ppm and 25 ppm) the stomatal frequency decreased while the stomatal index increased (Table 1). With higher concentration (50 ppm) there is complete absence of stomata (Table 1). With all the three treatments the epidermal cells enlarged. Some of the guard cells are highly stretched in width (Text-fig. 4) and a large number of persistent stomatal initials are also noticed.

6. 6-Ferfuryl aminopurine (Kinetin)—The results with the Kinetin treatment appears to be erratic (Table 1). However, the epidermal cell walls are slightly thickened (Pl. 2, Fig. 10).

7. 5-chloro-6-ethoxycarbonyl-methoxy-2,1,3-benzothiadiazole (Comp. II)—With 25 ppm of Comp. II there is a tremendous increase both in stomatal frequency and stomatal

index. While with all the three treatments, both the epidermal cells and guard cells become smaller (Table 1). Further the epidermal cell nuclei enlarge, guard cells become thick-walled (Pl. 2, Fig. 11) and many stomata are with unequal guard cells.

8. 2-chloroethyl phosphoric acid (CEPA)—With the low concentration of CEPA (10 ppm) both the stomatal frequency and stomatal index show a tremendous increase while both the epidermal cells and guard cells become smaller in size (Table 1). These departures from the control are less pronounced with higher concenrations of CEPA (50 ppm and 100 ppm). The epdermal cells and guard cells are slightly thick-walled (Pl. 2, Figl 12). A special feature is the frequent occurrence of cyclocytic stomata.

DISCUSSION

In genus *Vigna* paracytic type of stomata are known (METCALFE & CHALK, 1950). In *V. capensis* Shah (1968) and *V. ungniculata* Shah & Gopal (1968) reported haplocytic, anisocytic and anomocytic stomata in addition to paracytic ones. In the present study, in *V. sinensis*, I have not observed haplocytic stomata but cyclocytic and transitional stomata are found as additional types. Further, in paracytic and anisocytic types the ontogeny of stomata is both mesogenous and mesoperigenous, whereas, SHAH (1968) and SHAH AND GOPAL (1968) have observed only mesogenous development for paracytic and anisocytic types of stomata in *V. capensis* and *V. ungniculata*. I have found that the development of anomocytic stomata is aperigenous and that of cyclocytic stomata is cycloperigenous.

GANGADHAR AND INAMDAR (1975) studied the action of growth regulators on ontogeny of cotyledonary stomata of *Cucumis sativus*. They observed an increase in stomatal frequency in GA (50 ppm), IAA (50 ppm and 100 ppm) and COUM (50 ppm). In the present study, however, I have observed the low stomatal frequency in all these treatments. On the other hand, my observations regarding stomatal index are consistent with those of GANGADHAR AND INAMDAR (1975). Again, I found only a slight decrease in stomatal frequency and moderate increase in stomatal index in COL treatments, whereas GANGAHDAR AND INAMDAR (1975) have recorded tremendous decrease both in stomatal frequency and stomatal index. The formation of persistent stomatal initials are noticed in COL treatment as reported in *Dianthus caryophyllus* (GUYOT *et al.*, 1968) and *Abelmoschus esculantus* (INAMDAR, 1970). An interesting feature observed with 2,4-D treatment is that cyclocytic stomata are quite frequent instead of paracytic ones. Also, the various growth regulators used in present study effect the stomatal frequency, stomatal index, size of guard and epidermal cells unlike the report of TAL AND IMBER (1971).

However, it appears that growth regulators in general do not have an effect on ontogeny of stomata, which seems to be a genetic character as also noted by SUBRAHMANYAM *et al.*, (1974), SHAH *et al.*, (1974) and SHARMA AND DUNN (1968). But growth regulators have a marked effect on morphology of stomata. Furthermore, in the present study, I have observed the formation of greater number of cyclocytic stomata in 1 ppm 2,4-D and in various conentrations of CEPA. This phenomenon is a clear indication that due to the application of certain growth regulators the frequency of certain types of stomata, though already present in untreated material, may increas or decrease.

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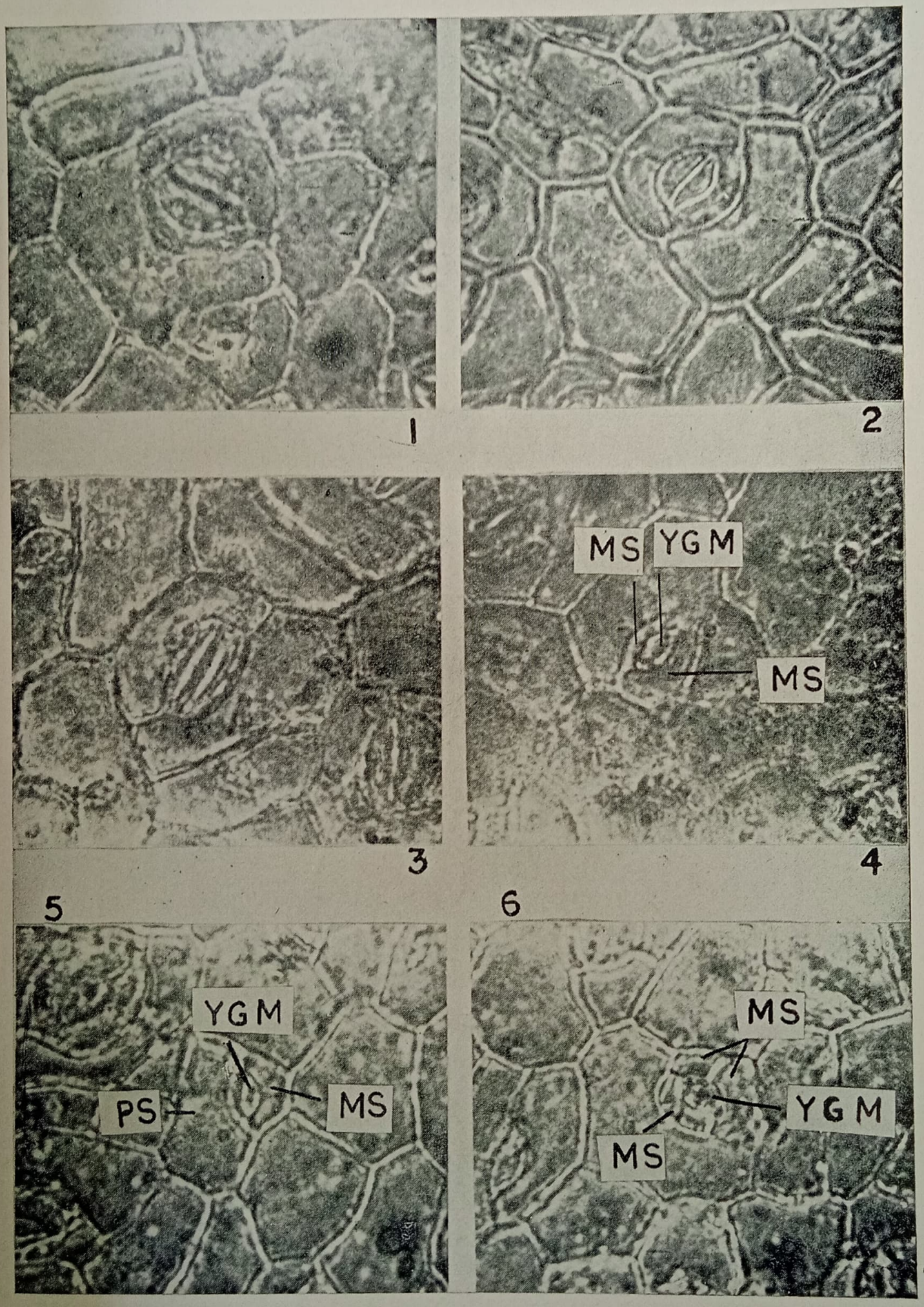
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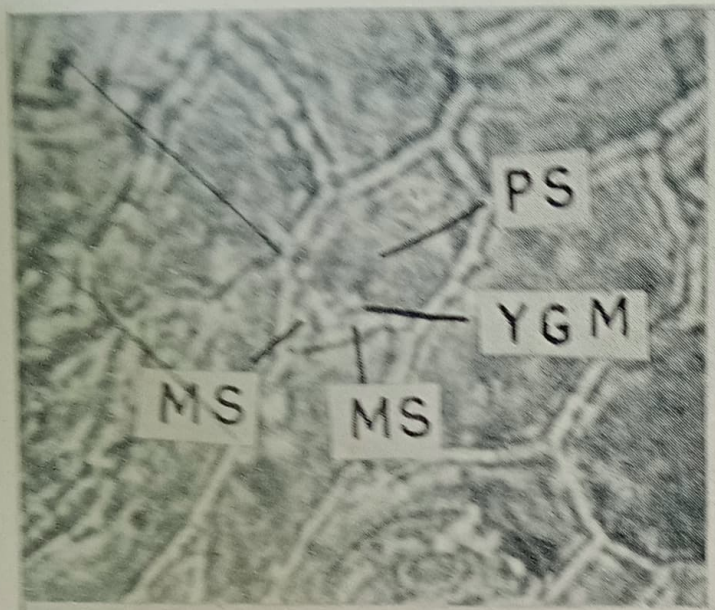

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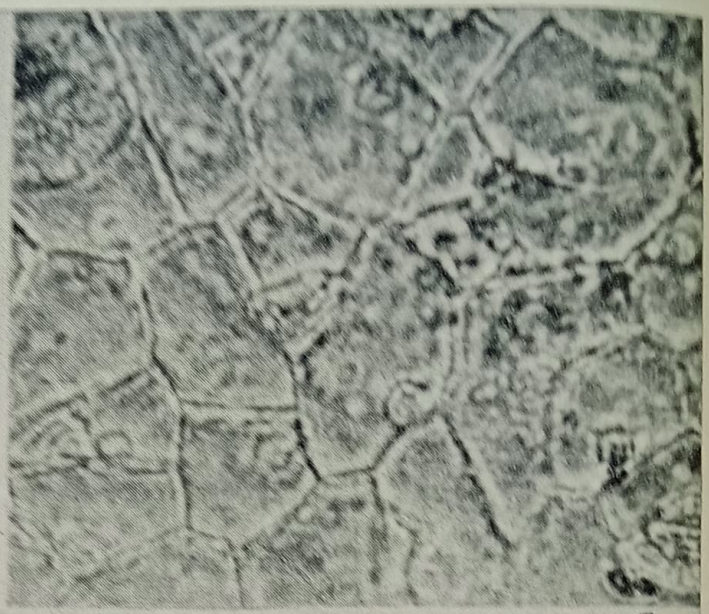

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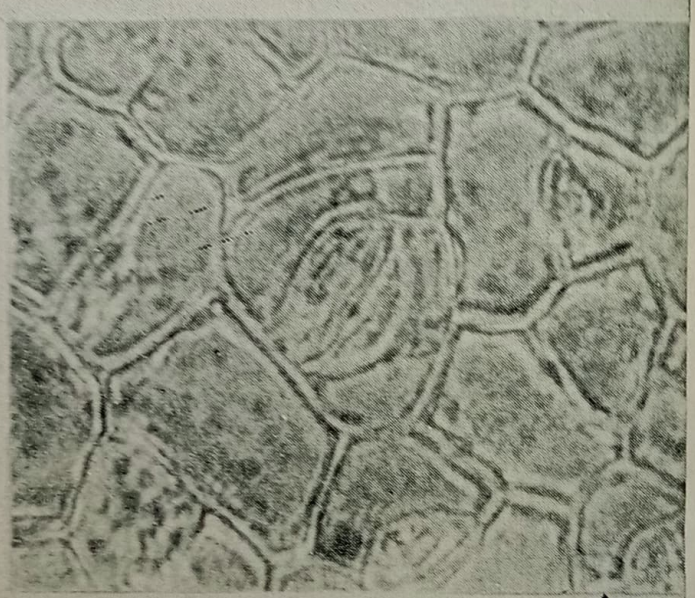

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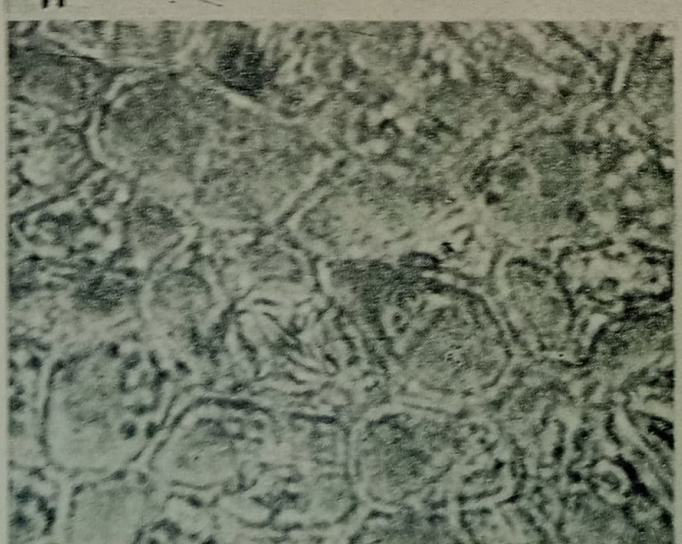

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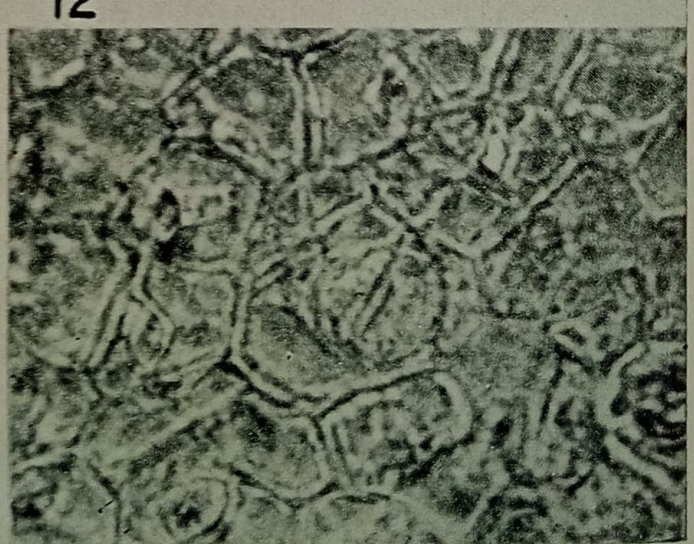

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EXPLANATION OF PLATES

PLATE 1

1. Paracytic stoma × 1000
2. Anisocytic stoma × 800
3. Transitional stoma × 1000
4. Paracytic stoma showing mesogenous development × 1000
5. Paracytic stoma showing mesoperigenous development × 1000
6. Anisocytic stoma showing mesogenous development, the young guard cell mother cell surrounded by recently divided three subsidiary cells × 1000

PLATE 2

7. Anisocytic stoma showing meosperigenous development, two subsidiary cells develop mesogenously and one develops perigenously × 1000.
8. Young meristemoid directly divides in to two guard cells giving anomocytic stoma aperigenously × 1000
9. The epidermal cell walls are slightly thick in GA × 800.
10. The thickened epidermal cell walls due to kinetin × 800.
11. The guard cells showing thick walls in Comp. II. × 800
12. The epidermal cells and guard cells are slightly thick walled in CEPA × 800

(The microphotographs of × 800 magnification are from different treatments and of × 1000 magnification are from control. MS= Mesogenously developed subsidiary cells ; PS= Perigenously developed subsidiary cells ; YGM= Young guard cell mother cell).