Proc. Indian Acad. Sci. (Plant Sci.), Vol. 95, No. 1, August 1985, pp. 21–28.

The interrelationship of growth substances and stomatal characters in *Gossypium hirsutum* var H–777

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MS received 13 September 1984

Abstract. Various types of stomatal abnormalities like persistent stomatal initials, single guard cells, degenerated guard cells and contiguous stomata with contiguity in different directions were recorded due to the treatment of different growth substances. Growth substances also altered the stomatal size, epidermal cell size, stomatal frequency, index and length-to-breadth ratio. It seems that the effect of these substances starts from the stomatal ontogeny and continue up to the last phase of development leading to such variations.

Keywords. Stomatal character; *Gossypium hirsutum*; growth substances

1. Introduction

Stomatal shape and arrangement on the leaf lamina are influenced by growth substances (Subrahmanyam *et al* 1972; Kasat 1979). These changes in stomata may be useful for a plant to grow in an unsuitable environment as stomata are the main outlets for water loss. Therefore, it is necessary to screen the growth substances for the purpose. In the present study, efforts have been made to find out the effects of 2,4,5-T, GA_3, ascorbic acid and 2,3,5-T on stomatal characters in *Gossypium hirsutum* var H-777.

2. Materials and methods

Experiments to find out the effects of various concentrations of 2,4,5-trichlorophenoxy acetic acid, ascorbic acid (AA), gibberellic acid (GA_3) and 2,3,5-triiodobenzoic acid (TIBA) on stomatal characters in *Gossypium hirsutum* var H-777, the seeds of which were procured from Hissar Agricultural University, Hissar, were performed in the Botanical Garden of the University. The plants were raised in unglazed earthenware pots of twelve inch diameter filled with a mixture of garden soil and farmyard manure (2:1 ratio) and were kept under natural conditions of light and temperature. Each selected concentration of a growth substance was sprayed on 10 plants. Thus the experimental design consisted of seventeen sets each with 10 pots. Aqueous solutions having 50, 100, 200 and 500 mg/l of AA, GA_3 and TIBA and 10, 25, 50 and 100 mg/l of 2,4,5-T were prepared. In all these solutions and distilled water, 2 ml of soapnut extract were added as wetting agent. The plants were sprayed when they were 1 month old having six leaves. It was repeated later 3 times at 10-day intervals. Mature leaves which emerged after the foliar spray treatments, were plucked, washed and fixed in FAA. Peels

of abaxial and adaxial sides were taken out from the lower, middle and apical regions of leaves. They were stained with safranin and temporary glycerine mounts were prepared.

Stomata of exact shape and size were drawn using camera lucida. Frequencies of stomata and epidermal cells per mm^2 were calculated. Length and breadth of stomata and epidermal cells were also measured. Stomatal index was calculated as defined by Salisbury (1927, 1932) *viz* $100S/(E+S)$, where E is the number of epidermal cells and S is the number of stomata over a given surface per unit area. Each reading is an average of 10 plants in a single treatment. To find out the relative effect of growth substances on length and breadth of the stomata, their ratio was calculated separately for both the surfaces.

3. Observations

Plants which were sprayed with 100 ppm 2,4,5-T and 500 ppm TIBA died. In the rest of the treatments, plants continued to grow.

(i) *Effect on epidermis*: The shape of the epidermal cells varies. It is either rectangular, polygonal, isodiametric or elongated. Cell walls are mostly straight (figure 1J) and sinuous (figure 1A).

(ii) *Effect on stomata*: Leaves are amphistomatic; stomata are randomly oriented throughout the epidermis. Majority of stomata are anomocytic type (figure 1A). In this type the epidermal cells surrounding the stoma are four in number and equal in size. They are so oriented that two epidermal cells lie parallel and two perpendicular to stoma. Some diacytic (figure 1B) and anisocytic (figure 1C) stomata were also observed. Following variations were observed in stomata in the control and treated leaves.

Persistent stomatal initials were encountered in TIBA 200 ppm (figure 1F). They showed slightly thicker wall with dense cytoplasm. Stomata with one guard cell only, were observed in GA_3 200 ppm and 500 ppm and 50 ppm 2,4,5-T (figure 1D). In GA_3 500 ppm, in some stomata, guard cells were found degenerated leaving only a thickening around the pore (figure 1I). In GA_3 200 and 500 ppm treatments, unequal guard cells, one being bigger and the other smaller, were also observed (figure 1H). Variously oriented contiguous stomata like juxtaposed (GA_3 200 and 500 ppm, figure 1K), superimposed (TIBA, 200 ppm; figure 1G) and at right angles to each other (TIBA 200 ppm, figure 1E). Stomata with abnormal big pore were recorded in 200 ppm GA_3 (figure 1L).

(iii) *Effect on epidermal cell size* (table 1): The epidermal cells present on abaxial and adaxial sides had a dimensions of $49{\cdot}95 \times 35{\cdot}80\ \mu$ and $48{\cdot}82 \times 37{\cdot}33\ \mu$ respectively. In 50 ppm 2,4,5-T, the epidermal cell size decreased while in ascorbic acid and TIBA, the size increased. GA_3 increased the epidermal cells in length.

(iv) *Effect on stomatal size* (table 1): In a normal leaf, the stomata of the adaxial side are bigger than those of abaxial side. 2,4,5-T, GA_3 and ascorbic acid treatments, increased the stomatal size on both the leaf surfaces. In TIBA treatment, stomata remained smaller in comparison to those of control.

(v) *Effect on frequency of epidermal cells* (table 2): The number of epidermal cells in a mm^2 of a normal leaf is 135·59 on abaxial side and 123·35 on the adaxial side. This frequency was increased by 2,4,5-T treatment only. The rest of the growth substances

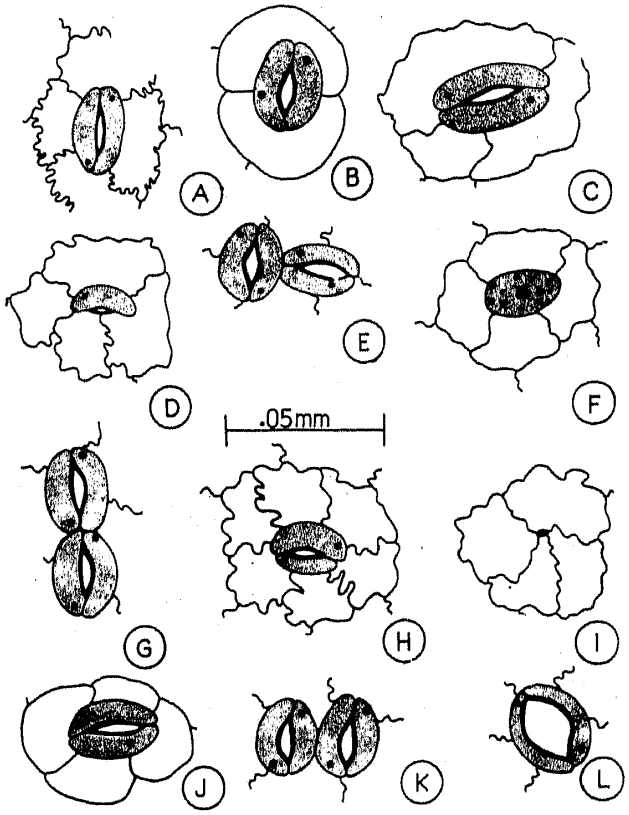

Figure 1. A–L. Effect of growth substances on the stomata of *Gossypium hirsutum* var H-777

used i.e. ascorbic acid, GA_3 and TIBA decreased the frequency of epidermal cells on both the leaf surfaces. The maximum decrease was in GA_3.

(vi) *Effect on frequency of stomata* (table 2): In control leaf, the stomatal frequency was 40·49 on the abaxial surface and 30·13 on the adaxial surface. Except TIBA all the growth substances used in the study increased the stomatal frequency.

(vii) *Effect on stomatal index* (table 2): This index comes out 22·99 and 19·63 on the abaxial and adaxial sides of control leaf. However, its value for both abaxial and adaxial surfaces was more than these in all the treatments except TIBA.

(viii) *Effect on stomatal length/breadth ratio* (table 1): This ratio shows that stomata were broadly elliptic on both surfaces of a normal leaf. They were broadly elliptic on the abaxial side in 10, 25 and 50 ppm 2,4,5-T but were narrow elliptic on the adaxial side. In 50 ppm and 500 ppm AA, stomata were broadly elliptic but were narrow elliptic in 100 and 200 ppm ascorbic acid on the abaxial side. On the adaxial side, they were narrow elliptical in 50, 100 and 200 ppm ascorbic acid and broadly elliptic in 500 ppm. In GA_3, stomata appeared narrow elliptic on the abaxial side of 200 and 500 ppm treatments and on the adaxial side of 200 ppm. In the rest of the concentrations of GA_3, the stomata were broadly elliptic on both the leaf surfaces. Broadly elliptic shape of the stomata remains unchanged on both surfaces of the leaf in TIBA except on the abaxial side in 100 ppm where the stomata become narrow elliptic.

4. Discussion

Growth substances have affected the shape and size of stomata and epidermal cells variously. In the present investigation, abnormalities like persistent stomatal initial,

Table 1. Effect of different growth substances on epidermal characters in *Gossypium hirsutum* var H-777

Treatment	Concentration (ppm)	Epidermal cell size (μ)				Stomatal size (μ)				Length/breadth Ratio*	
		Abaxial		Adaxial		Abaxial		Adaxial			
		Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Abaxial	Adaxial
Control	0	49·95 ±3·24	35·80 ±2·04	48·82 ±3·23	37·73 ±0·89	29·97 ±0·84	19·98 ±4·31	31·63 ±6·32	21·64 ±4·23	1·49	1·46
2,4,5-T	10	47·45 ±1·27	33·30 ±1·08	43·29 ±4·02	37·63 ±0·75	29·97 ±1·87	20·81 ±2·81	35·96 ±5·43	23·31 ±3·21	1·44	1·54
	25	39·96 ±2·04	34·10 ±1·07	43·29 ±5·01	29·64 ±0·76	33·00 ±2·83	21·98 ±3·91	38·96 ±4·23	24·14 ±2·63	1·58	1·61
	50	39·13 ±1·09	30·80 ±3·24	36·63 ±4·02	30·64 ±0·83	32·47 ±1·87	22·48 ±2·06	36·30 ±3·24	22·48 ±2·23	1·44	1·62
	100	–	–	–	–	–	–	–	–	–	–
AA	50	49·95 ±5·72	36·63 ±0·94	46·62 ±3·01	39·96 ±0·75	29·97 ±2·34	21·65 ±2·08	35·96 ±3·23	19·98 ±3·21	1·38	1·80
	100	53·28 ±6·03	35·79 ±0·24	49·12 ±4·02	43·29 ±0·76	33·30 ±4·32	21·65 ±3·07	36·63 ±4·24	22·48 ±2·23	1·54	1·63
	200	52·45 ±4·02	42·45 ±3·24	49·95 ±5·07	42·29 ±0·83	39·13 ±7·52	20·81 ±0·94	42·29 ±1·23	26·64 ±3·32	1·88	1·59
	500	53·28 ±3·04	49·45 ±4·27	53·28 ±6·02	43·29 ±0·75	34·13 ±2·24	23·31 ±0·74	36·29 ±2·21	24·97 ±2·19	1·46	1·45

GA_3	50	59·94 ±2·09	26·64 ±2·63	53·28 ±2·03	29·97 ±0·82	29·97 ±3·32	19·98 ±1·24	28·30 ±2·32	23·31 ±2·18	1·50	1·21
	100	79·92 ±0·92	29·97 ±1·62	52·54 ±4·09	28·97 ±2·34	32·63 ±3·12	22·48 ±1·23	33·30 ±3·32	25·80 ±2·62	1·45	1·29
	200	73·26 ±0·56	23·31 ±0·94	53·28 ±5·02	29·97 ±2·94	36·63 ±4·06	21·64 ±1·22	36·63 ±3·43	23·31 ±3·12	1·69	1·57
	500	69·93 ±0·43	36·63 ±0·93	56·61 ±3·02	36·63 ±1·63	36·09 ±5·02	22·98 ±2·22	35·23 ±4·42	26·64 ±3·14	1·57	1·32
TIBA	50	53·28 ±0·42	33·30 ±0·94	48·47 ±4·02	38·63 ±0·92	29·13 ±4·05	19·98 ±1·06	29·97 ±2·39	19·98 ±1·08	1·46	1·50
	100	49·62 ±0·82	39·30 ±0·93	53·29 ±5·03	38·80 ±3·73	25·97 ±3·06	19·18 ±1·05	26·64 ±2·64	19·98 ±1·02	1·36	1·33
	200	53·29 ±0·93	38·30 ±0·72	50·79 ±4·32	36·46 ±4·62	24·97 ±2·05	14·15 ±2·07	23·31 ±3·62	19·98 ±2·07	1·76	1·17
	500	–	–	–	–	–	–	–	–	–	–

* If equal to or more than 1·5 = broad elliptical. If less than 1·5 = narrow elliptical.

Table 2. Effect of different growth substances on epidermal characters in *Gossypium hirsutum* var H-777

Treatment	Concentration (ppm)	Frequency of epidermal cells (mm^{-2})		Frequency of stomata (mm^{-2})		Stomatal index	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Control	0	135·59 ±15·29	123·35 ±20·20	40·49 ±4·32	30·13 ±2·08	22·99	19·63
2,4,5-T	10	131·83 ±20·87	129·94 ±21·22	49·34 ±3·56	35·78 ±1·07	27·23	21·59
	25	154·43 ±10·56	137·48 ±13·33	54·24 ±7·32	42·75 ±1·05	25·99	23·72
	50	148·77 ±9·82	131·83 ±14·32	56·49 ±8·26	45·19 ±1·05	27·52	25·53
	100	–	–	–	–	–	–
AA	50	133·71 ±20·75	120·53 ±20·22	46·70 ±3·25	33·89 ±2·04	25·89	21·95
	100	123·73 ±10·42	123·73 ±16·72	48·96 ±4·32	37·66 ±0·92	28·35	23·07
	200	124·29 ±10·42	125·61 ±14·12	53·11 ±3·23	40·87 ±2·02	29·94	27·23
	500	118·64 ±5·82	99·81 ± 8·07	54·61 ±3·25	39·36 ±1·98	31·52	28·28
GA_3	50	130·32 ±20·75	101·69 ±20·92	50·85 ±4·75	38·98 ±1·78	28·06	27·71
	100	97·36 ± 6·56	92·28 ±7·58	48·59 ±6·78	45·20 ±1·72	33·16	32·88
	200	99·81 ±8·98	79·09 ±6·02	48·96 ±8·89	41·43 ±1·73	32·91	34·58
	500	90·39 ± 7·08	74·76 ±8·09	50·85 ±9·35	47·46 ±0·96	35·66	36·83
TIBA	50	133·71 ±15·74	120·03 ±20·02	36·16 ±4·30	35·22 ±2·32	21·29	20·68
	100	120·53 ±13·24	112·99 ±18·75	25·80 ±2·20	20·53 ±4·01	17·63	15·37
	200	105·27 ±12·64	118·64 ±20·02	19·40 ±1·05	22·03 ±3·32	15·56	15·66
	500	–	–	–	–	–	–

single guard cell which according to Inamdar *et al* (1974) develop either directly from meristemoid or by degeneration of one of the guard cells, degenerated guard cells and contiguous stomata in which contiguity was in different directions, were observed on both the surfaces of the leaf in *Gossypium hirsutum* var H-777 due to treatment with various doses of growth substances. These findings support the earlier findings of Inamdar *et al* (1974) who also observed abnormalities in stomatal shape due to treatment of growth substances. Various explanations like cytoplasmic heterogeneity (Morgan 1934), extrinsic factors (Bunning 1952), intrinsic instability (Dehnel 1961), genetic factor (Kasat 1979; Sharma and Dunn 1968) have been given for the formation of abnormal stomata.

As a general effect, 2,4,5-T, GA_3 and ascorbic acid increased the stomatal size, index

and frequency on both the surfaces of leaf in *G. hirsutum*. However, TIBA, applied at higher concentrations, reduced stomatal size, frequency and index on both the surfaces. Earlier Gangadhara and Inamdar (1975) observed increased stomatal frequency in GA and IAA treated *Cucumis sativus* seedlings.

Owing to smaller size of epidermal cells in 2,4,5-T treatment, the frequency of these cells increased. However, ascorbic acid and TIBA increased the size of epidermal cells and thus their frequency declined. Similar was the effect of GA_3 and these findings agree with Murty *et al* (1976) who also observed enlargement of epidermal cells due to GA treatment.

Ratio of stomatal length to breadth which was calculated to find out the effect of different growth substances on stomatal shape showed that in *G. hirsutum*, the stomata are broadly elliptic on both surfaces of a control leaf and remain the same on the abaxial surface in 10 and 50 ppm 2,4,5-T, 50 and 500 ppm ascorbic acid, 50 and 100 ppm of GA_3 and TIBA, but they became narrow elliptic in 25 ppm 2,4,5-T, 100 and 200 ppm ascorbic acid, 200 and 500 ppm GA_3 and 200 ppm TIBA. On adaxial surface they were broadly elliptical in control, 500 ppm AA, all concentrations of GA_3 and TIBA but became narrow elliptic in the rest of the treatments.

It is clear from the present discussion that growth substances produce several variations in the morphology of stomata as well as in their frequency, index etc, which are possible only if the ontogeny was affected. Therefore, the findings support Gangadhara *et al* (1977) who was of the opinion that differentiation of a meristemoid into stomatal complex may be controlled by the specific intrinsic factors at the region of meristemoid and these factors are disturbed by the exogenous application of growth substances which ultimately may lead to such aberrant developments. The view of Kasat (1979) who considered that growth substances in general do not have any effect on ontogeny of stomata but they affect their morphology in the last phase of their development did not get any support from the present findings except that the morphology would have been altered at the later phase of their development leading to variations in stomatal shape.

Acknowledgements

One of us (RKS) is thankful to CSIR, New Delhi for the award of a fellowship.

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