Interactions of Microtubule Disorganizers, Plant Hormones, and Red Light in Wheat Coleoptile Segment Growth[1]

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ABSTRACT

Growth response of coleoptile segments excised from 3-day-old seedlings of wheat (*Triticum vulgare* cv. Baart) to gibberellic acid, indoleacetic acid, and 2,4-dichlorophenoxyacetic acid, to red light, and to several microtubule disorganizers depends on the initial position of the excised segment in the intact coleoptile. Red light, 660 nm, stimulates the growth of the apical cells, but inhibits markedly the growth of the cells in the basal region of the coleoptile. The effects of red light are independent of sucrose, gibberellic acid, indoleacetic acid, and 2,4-dichlorophenoxyacetic acid, even though these substances themselves markedly affect the growth of the coleoptile segments. Concentrations of the microtubule disorganizers, vinblastine sulfate, cupric chloride, urea, and colchicine, which do not alter significantly the growth of the dark control apical segments, substantially repress the promotive effects of red light or auxin on the increase in length of the apical cells of the coleoptile. This suggests that stimulation by red light and by auxin involves microtubule production. Microtubule disorganizers repress the growth of elongating cells of the coleoptile, yet on the other hand, auxin and irradiation do not alter significantly the response of basal cells to the microtubule disorganizing agents. We hypothesized that light and growth regulators induce polymerization of nonaggregated microtubule subunits, resulting in faster growth.

Excised segments of coleoptiles have been used extensively as experimental test material in growth regulator studies since 1933, when Bonner indicated that coleoptile explants would continue to grow in an incubation medium. Most investigators have employed a subapical 2- to 15-mm (mostly 8- to 10-mm) oat or wheat segment excised from the apical half of the coleoptile excluding the most apical 3 mm. Only a few studies dealing with the apical segment itself have been performed.

In most of the oat experiments, red light has been used either to suppress mesocotyl development, to perform manipulations, or to serve as growth lighting throughout the incubation period. Under such conditions, any interpretation of the effect of growth regulators on coleoptile growth would be difficult. Not only is the interaction of the mesocotyl and coleoptile in oat development not thoroughly understood but it has also been documented that red light affects growth of oat coleoptile subapical segments. In any consideration, therefore, of the effects of auxin or GA_3 on growth of coleoptile segments exposed to red light, the effects of red light itself would first have to be ascertained. Only then could the induced growth due to the growth regulators be estimated. In virtually all of these previous studies, such an estimation would be impossible.

Also, no systematic study of the effects of auxin and GA_3 on the growth of serial explants cut from dark-grown coleoptiles had been previously reported. It seemed essential to perform such a test prior to initiating studies dealing with the interactions of auxin and GA_3 with other growth-regulating agents. The use of a green safelight for manipulations and dark incubation period before and after the excision of segments from the intact coleoptile was standard operations for such tests.

That red light is a regulatory agent in coleoptile cell growth has been well documented; however, there is disagreement as to the nature of the effect of red light on the growing zone of the coleoptile. Some investigators report a stimulatory effect of red light on the coleoptile segments cultured with and without auxins (1, 9, 12, 17) while others note no additional growth (8, 10). One investigation on excised segments cultured in a solution of sucrose and maleate shows a slight repressive effect due to irradiation with red light (5). In view of these results, it seemed essential to determine the effects of red light on growth of segments excised from different positions on the intact coleoptile and cultured in various media.

It has been established that plant cell growth involves cell wall synthesis which in turn involves deposition of cellulose microfibrils. Microtubules, cytoskeletal units which are affected by environmental stresses, have been assigned a role in governing microfibril deposition in the plant cell wall (11, 13–15). Agents which are known to disrupt microtubules and prevent polymerization of subunits into formed microtubules are colchicine (13, 19, 20), vinblastine sulfate (2, 4), urea (18), and the cupric ion (16). Since microtubules play a key regulatory role in cell elongation, it seemed interesting to determine if the additional growth due to exogenous auxin and irradiation could be prevented by agents which disrupt microfibrils. The assumption would be that colchicine, vinblastine sulfate, urea, and the cupric ion would interfere with any cell function dependent on an organized microtubule array.

MATERIALS AND METHODS

Unimbibed grains of wheat (*Triticum vulgare* cv. Baart) were sown broadcast on tap water-saturated Kimpak[3] contained in

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[3] Kimpak is an absorbent cellulose material supplied by Kimberly-Clark Manufacturing Co.

shallow metal trays. The trays were enclosed in dark cabinets in a dark room maintained at 25 to 26 C. Pans of water were placed in the cabinets to maintain a minimum relative humidity of 65%.

Test plants were selected and manipulations were performed under a green safelight at low intensities. The safelight was constructed of a 15-w green fluorescent tube wrapped with duPont yellow cellophane and No. P-42 bluegreen plastic film (Gelatine Products, Brooklyn, New York). The emission spectrum of the safelight was 490 to 550 nm, with a peak emission at 520 to 525 nm. Exposure of the plants was kept as brief as possible even though a preliminary trial showed that the green safelight did not alter the subsequent elongation of the coleoptile.

By means of a multibladed cutter using razor blades, segments were excised from straight coleoptiles of a uniform length 30 ± 5 mm. By the use of 5-mm spacers, it was possible to cut segments of a predetermined length and position. The apical 20 mm of the intact coleoptile was cut into four 5-mm segments. These segments were designated a, b, c, and d in descending order from the tip of the coleoptile. The enclosed leaf was not removed from the coleoptile, since preliminary experiments showed no effect of the presence of the leaf on the response to test solutions.

Segments in groups of 25 replicates were incubated in 20 ml of test solution in 50-ml beakers in the same dark cabinets used for culture of the seedlings for 23 to 24 hr. Concentrations of microtubule disorganizers used in the experiments were chosen on the basis of two criteria: a) concentrations which did not significantly alter the growth of the a segment as compared to sucrose, and b) concentrations which would give a similar response in the d segment with respect to sucrose.

RESULTS

Segment Response to Growth Regulators. The response of coleoptile segments to growth regulators varies with the initial position of the segment in the intact coleoptile as well as with the type of growth regulator used. Growth in all test solutions is greatest in the subapical b segment. Minimal growth is achieved in the apical a segment in H_2O as well as in solutions of either sucrose or GA_3. However, in IAA or 2,4-D, growth is least in the d segment. Growth in sucrose is approximately twice as great as that in H_2O for all segments. Although the over-all effects of GA_3 and auxin were approximately the same, the two types of growth regulators do differ in their effects on particular segments (Table I).

Indole-3-acetic acid and 2,4-D can be used interchangeably as growth promoters (Table I). The optimal concentration of auxin for growth promotion of the a segment is quite sharp at 0.1 mM; however, the other segments do not exhibit a clear optimal concentration up to 0.2 mM. The concentration-growth curve for these segments is quite flat (Fig. 1). Although one might expect an increase in growth of b and c segments upon the addition of IAA relative to that observed in sucrose, such an increase did not occur in the series of experiments carried out with Baart wheat. One possible explanation for this is that IAA is not the limiting factor in the elongation of the b and c segments.

GA_3 in the concentration range 1 to 50 μM supports optimal growth of all segments (Fig. 2).

Segment Response to Irradiation. Exposure to red light, 660 nm, for 3 min prior to a 24-hr incubation period affects growth of various types of segments quite differently. Relative to the growth of the dark controls, irradiation promotes growth of the a segment, has little or no effect on growth of the b segment, and inhibits growth of the c and d segments. Growth response to irradiation, therefore, depends on the type of segment used. The promotive or inhibitory effect of red light on segment growth occurs independently of the type of growth regulator in the incubation medium, although sucrose, auxin, and GA_3 themselves affect markedly the absolute amount of growth (Table I).

Segment Response to Microtubule Disorganizers. Colchicine, at concentrations up to 10 mM, does not alter significantly the growth of apical segments incubated in the dark for 24 hr in a sucrose medium (Fig. 3, Table II). Vinblastine sulfate (0.2 mM), $CuCl_2$ (0.1 mM), and urea (0.4 M) also do not markedly affect the dark growth of the coleoptile segments incubated in a sucrose medium (Table III).

The basal d segment response to colchicine is quite different

Table I. *Effect of Medium Constituents on Growth of Excised Wheat Coleoptile Segments*

Initial length of coleoptile was 30 ± 5 mm and the initial length of segments was 5 mm. The growth period was 24 hr. Data are means from 20 different experiments.

Medium	Increase in length of segments											
	a			b			c			d		
	D[1]	R[2]	R/D[3]	D	R	R/D	D	R	R/D	D	R	R/D
						mm						
Water	1.9 ± 0.20	3.8 ± 0.22	2.01	4.5 ± 0.18	4.7 ± 0.24	1.05	4.2 ± 0.22	3.8 ± 0.20	0.89	2.7 ± 0.17	2.1 ± 0.20	0.77
Sucrose, 50 mM	2.7 ± 0.17	4.7 ± 0.20	1.76	9.5 ± 0.12	9.4 ± 0.14	0.99	8.2 ± 0.20	6.9 ± 0.18	0.84	5.8 ± 0.20	3.2 ± 0.22	0.56
Sucrose, 50 mM + GA₃, 10 μM	3.8 ± 0.26	6.5 ± 0.22	1.73	11.1 ± 0.28	11.8 ± 0.30	1.06	10.4 ± 0.30	8.6 ± 0.28	0.82	7.3 ± 0.28	4.2 ± 0.30	0.58
Sucrose, 50 mM + IAA, 0.1 mM	5.9 ± 0.30	8.8 ± 0.26	1.49	10.0 ± 0.12	10.4 ± 0.16	1.04	7.9 ± 0.26	6.9 ± 0.28	0.85	5.1 ± 0.22	3.9 ± 0.24	0.77
Sucrose, 50 mM + 2,4-D, 0.1 mM	5.7 ± 0.17	7.9 ± 0.20	1.40	10.2 ± 0.22	10.2 ± 0.20	1.00	7.8 ± 0.24	6.8 ± 0.26	0.86	5.4 ± 0.17	4.3 ± 0.20	0.79

[1] Increase in length of nonirradiated segments.

[2] Increase in length of segment floated on indicated media, exposed 3 min to 660 nm radiation and returned to darkness for 24 hr.

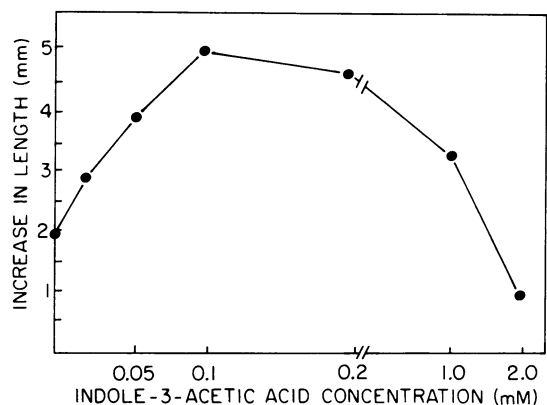[3] R/D: increase in length of irradiated/increase in length of nonirradiated.

FIG. 1. Effect of indole-3-acetic acid in a solution of 50 mM sucrose on the growth of wheat coleoptile *a* segments.
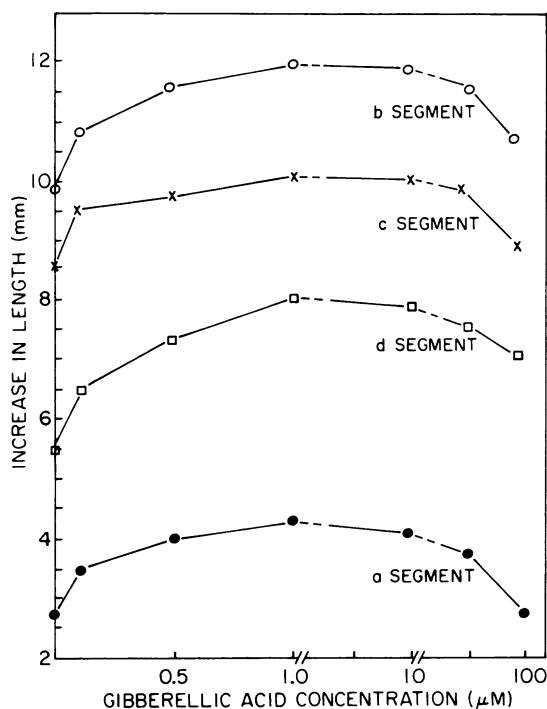

FIG. 2. Effect of gibberellic acid in a 50 mM sucrose solution on the growth of wheat coleoptile segments.

from that of the apical segment. As the concentration of colchicine increases from 0.02 to 10 mM, the per cent inhibition due to the compound increases by essentially the same amount in both irradiated and nonirradiated segments.

The effects of microtubule disorganizers on the *b* and *c* segments were not studied, since the response of these segments to IAA and red light differs only slightly from their response to sucrose.

Effects of Microtubule Disorganizers on Irradiation Response of Segments. The absolute amount of increase in length of the apical segment due to irradiation decreases as the concentration of colchicine increases (Fig. 3, Table II). Concentrations of vinblastine sulfate, cupric chloride, urea, and colchicine, which do not significantly affect the dark growth of the control apical segment, repress about three-fourths of the promotive effects of irradiation on growth of the apical segment (Table III).

Colchicine causes slight to no change in the response of basal segments to irradiation (Fig. 3, Table II). Vinblastine sulfate, cupric chloride, and urea behave in a similar manner. Test

solutions which decrease dark growth of the basal segment by approximately one-third actually repress growth of irradiated segments by only 10% (Table IV).

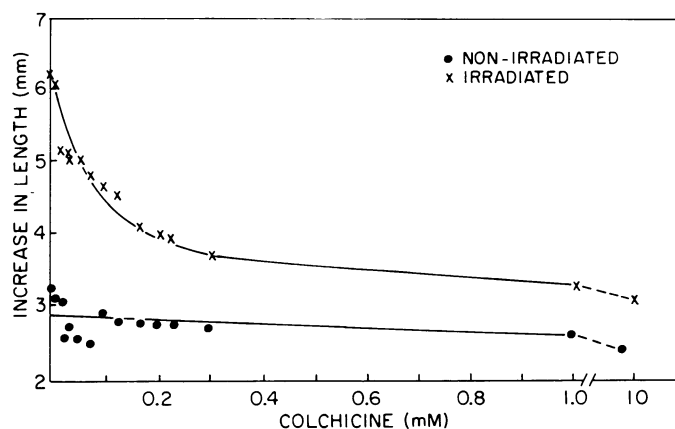Colchicine (0.1 mM) supplied to the apical segment prior to

FIG. 3. Effect of colchicine in a 50 mM sucrose solution on growth of irradiated and nonirradiated excised apical coleoptile segments.

Table II. *Effect of Colchicine Supplied before or after Irradiation on Growth of Coleoptile Segments*

Initial coleoptile length was 30 ± 5 mm, and the initial length of segments was 5 mm. The medium was 50 mM sucrose. Irradiation was a 3-min exposure to 660 nm. The growth period was 24 hr.

Segment	Colchicine Concn in Medium, 0.1 mM	Increase in Length	
		Nonirradiated	Irradiated
		mm	
a	None	2.9 ± 0.33	6.2 ± 0.50
	30-sec preirradiation	3.0 ± 0.13	4.1 ± 0.20
	5-min postirradiation		5.0 ± 0.37
	30-min postirradiation		5.2 ± 0.34
	60-min postirradiation		5.1 ± 0.25
d	None	5.5 ± 0.37	3.3 ± 0.10
	30-sec preirradiation	3.9 ± 0.24	3.0 ± 0.43
	5-min postirradiation		3.4 ± 0.17
	30-min postirradiation		3.1 ± 0.11
	60-min postirradiation		3.2 ± 0.27

Table III. *Suppression of Light Growth Stimulation of Apical Segment by Various Microtubule Disorganizers*

Initial coleoptile length was 30 ± 5 mm and 5-mm apical segments. The medium was 50 mM sucrose plus or minus disorganizing agents. The growth period was 24 hr. Irradiation was a 3-min. exposure to 660 nm. Data are means of five experiments.

Medium	Increase in Length		Repression due to Agent
	D[1]	R[2]	
	mm		%
Sucrose	2.9 ± 0.20	5.3 ± 0.22	Control
Sucrose + vinblastine sulfate (0.2 mM)	2.6 ± 0.22	3.6 ± 0.24	69
Sucrose + CuCl₂ (0.1 mM)	3.4 ± 0.30	4.4 ± 0.26	83
Sucrose + urea (0.4 M)	3.3 ± 0.28	3.8 ± 0.24	70
Sucrose + colchicine (0.1 mM)	3.4 ± 0.22	4.5 ± 0.24	84

[1] Nonirradiated segments.
[2] Irradiated segments.

irradiation, represses about two-thirds of the response to red light. This same concentration of colchicine, when added to the incubation medium of sucrose 5, 30, or 60 min following irradiation of the *a* segment, suppresses only one-third of the growth promotion due to red light.

The suppression of the growth of the basal segment due to colchicine is the same whether the test compound is added before or after irradiation (Table II).

Effect of Microtubule Disorganizers on Auxin-induced Growth. Vinblastine sulfate, urea, and $CuCl_2$ repress the induced growth of the apical segment due to auxin by 83, 83 and 100%, respectively. Colchicine, however, is less effective in blocking the action of auxin on the *a* segment, repressing the additional growth due to auxin by about one-third.

Repression of the growth of the basal *d* segment by the microtubule disorganizers is only slightly enhanced in the presence of auxin (Table V).

DISCUSSION

There has been disagreement among investigators about the effects of auxin, GA₃, and red light on the growth of coleoptile segments. This study was designed to determine the effects of these factors on segment growth response and the interaction of these growth factors with microtubule disorganizers.

The effects of IAA, 2,4-D, GA₃, and 660 nm red light on the growth of coleoptile segments depends upon the initial

Table IV. *Effect of Various Microtubule Disorganizers on Growth of Basal d Coleoptile Segments*

The initial coleoptile length was 30 ± 5 mm. The medium was 50 mM sucrose plus or minus disorganizing agent. The growth period was 24 hr. Irradiation was a 3-min exposure 660 nm. Data are means of 10 experiments.

Medium	Increase in Length		Repression due to Agent	
	D[1]	R[2]	D	R
	mm		%	
Sucrose	5.5 ± 0.23	3.2 ± 0.20	Control	
Sucrose + vinblastine sulfate (0.2 mM)	3.7 ± 0.22	2.9 ± 0.23	33	9
Sucrose + $CuCl_2$ (0.1 mM)	3.7 ± 0.20	2.8 ± 0.22	33	12
Sucrose + urea (0.4 M)	4.5 ± 0.19	2.7 ± 0.22	29	12
Sucrose + colchicine (0.1 mM)	3.7 ± 0.21	2.9 ± 0.24	32	10

[1] Nonirradiated segments.
[2] Irradiated segments.

position of the segment in the intact coleoptile. As compared with dark growth of the control segments in a solution of 50 mM sucrose, the absolute amount of growth due to auxin is greatest in the apical segment and decreases from the coleoptile apex basipetally. The effects of GA₃ are quite different. This growth regulator supports maximum growth in a zone 15 to 20 mm below the apex of the coleoptile. A comparison of the effects of GA₃ and auxin on dark growth of coleoptile segments shows that, except for the apical segment, GA₃ induces significantly more growth in the nonirradiated segments than does either IAA or 2,4-D. The ultimate effects of red light on segment growth depend entirely on what type of segment is irradiated. Whereas red light promotes growth of the apical segment, it has only a small effect on the *b* segment, and inhibits growth of the *c* and *d* segments. These effects occur both in the absence and in the presence of auxin or gibberellic acid. The same pattern of response to red light occurs even in a culture medium of distilled H_2O. Growth effects of auxin or GA₃ are produced in both irradiated and nonirradiated segments. It seems probable, therefore, that the response to red light does not depend upon changes in the endogenous levels of these growth regulators.

Enlargement of plant cells is accompanied by (perhaps dependent upon) synthesis of the primary wall which consists in large part of cellulose microfibrils. In cells such as those of the coleoptile which exhibit polarized elongation, the microtubules are oriented circumferentially. The microfibrils are similarly orientated. This arrangement offers minimal resistance to stretching (13).

Several agents, e.g. vinblastine sulfate, urea, colchicine, and the cupric ion, are known to inhibit microtubule formation as well as to cause the disaggregation of polymerized microtubules (2, 7, 16, 18). Growth stimulation due to auxin and red light is effectively diminished by the addition of the microtubule disorganizers. This seems to indicate that polymerization of microtubule subunits is required for the action of both auxin and red light. Some of the induced growth due to the latter two effectors is not eliminated by the presence of microtubule disorganizers. This lack of response to the action of the disorganizing agents is very apparent when the agents are added to the culture medium at various time intervals following irradiation. It is suggested that the dark control level of growth is due to a group of formed microtubules. Additional growth due to auxin and red light requires the polymerization of microtubule subunits. The presence of microtubule disorganizers in the incubation medium partially prevents this subunit polymerization. The additional growth due to auxin and red light, not eliminated by the disorganizer agents, suggests that via some mechanism, perhaps alteration of attachment sites of

Table V. *Effect of Microtubule Disorganizers on Auxin-induced Growth of Apical and Basal Coleoptile Segments*

The initial coleoptile length was 30 ± 5 mm and the initial segment length was 5 mm. The medium was 50 mM sucrose plus or minus the disorganizing agent and 0.1 mM IAA. The growth period was 24 hr. Data are means of five experiments.

Medium	Increase in Length of Segments				Repression due to Agent			
	a		*d*		*a*		*d*	
	−IAA	+IAA	−IAA	+IAA	−IAA	+IAA	−IAA	+IAA
	mm				%			
Sucrose + IAA	2.9 ± 0.10	7.1 ± 0.50	5.2 ± 0.12	5.0 ± 0.20	Control			
Sucrose + vinblastine sulfate (0.2 mM)	2.6 ± 0.26	1.2 ± 0.22	3.7 ± 0.44	3.2 ± 0.31	9	83	29	35
Sucrose + urea (0.4 mM)	2.7 ± 0.30	1.2 ± 0.40	3.9 ± 0.33	3.0 ± 0.11	8	83	25	40
Sucrose + $CuCl_2$ (0.1 mM)	2.5 ± 0.17	0.0	4.0 ± 0.27	3.0 ± 0.19	13	100	23	41
Sucrose + colchicine (0.1 mM)	2.7 ± 0.11	4.7 ± 0.23	3.7 ± 0.17	3.2 ± 0.20	6	33	29	36

the microtubule disorganizer to the subunit, a number of sub-units "escape" the action of the disorganizing agents and are free to polymerize and form microtubules. These, then, become responsible for the extra growth due to auxin and red light.

The fact that red light and auxin operate independently of each other suggests either that these two effectors each stimulate separate compartments of microtubule subunits, or that they affect the same compartment through separate mechanisms. Each effector gives rise to its own quota of formed microtubules resulting in enhanced growth of the coleoptile cell.

If polymerization of microtubule subunits is responsible for the enlargement of the coleoptile cell, a mechanism must then be provided to explain how red light represses growth of the basal segment but promotes growth of the apical segment, and auxin, on the other hand, has only a negligible effect on the growth of the basal segment but triples the growth of the apical segment. A suggested explanation for the effects of auxin and red light on the basal segment is as follows. As the cell enlarges, orientation of the microtubules changes from a strictly circumferential to a longitudinal arrangement, the latter resisting cell elongation in an axial direction. Although it is assumed that auxin and red light increase polymerization of subunits into formed microtubules at all stages of growth, the ultimate effect on growth will vary from promotion to ineffectiveness and even repression, depending on the stage of development of the cell.

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