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Red Light-inhibited Mesocotyl Elongation in Maize Seedlings

I. THE AUXIN HYPOTHESIS[1]

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ABSTRACT

Red light-inhibited mesocotyl elongation, which occurs in intact *Zea mays* L. seedlings, was studied in excised segments which included the coleoptile (or parts therefrom) and apical centimeter of the mesocotyl. Experiments took into account, first, the ability of the segments to regenerate auxin supply sites, and, second, that auxin uptake can be greatly reduced if there is no cut surface, apical to the elongating cells, to act as a port of entry. In all cases, auxin completely reversed the inhibition of elongation by light. The results support the hypothesis that light regulates mesocotyl elongation by controlling auxin supply from the coleoptile. Sucrose concentration had no effect on auxin reversal of light-inhibited elongation, but relatively high concentrations of gibberellic acid (10 μM) could substitute for auxin in this system.

The growth of a seedling germinating in the dark is affected by illumination in two important ways. One of these, phototropism, has been extensively studied and reviewed (*e.g.* 7). The second, light-inhibited shoot elongation, has been somewhat less studied, but is equally important in the economy of the plant. Such inhibition of shoot elongation has been known since 1894 (21). However, it was not until 1936, after the discovery of auxin, that a mechanism for this inhibition was proposed. Van Overbeek (28) suggested that light regulated mesocotyl elongation by controlling auxin supply from the coleoptile. He showed a significant reduction in production of diffusible auxin from coleoptile tips when the etiolated seedlings were exposed to 3 hr of room light. The cells containing the photoreceptor would thus be remote from the responding tissue. His proposal was supported by Inge and Loomis (11) who antidoted white light inhibition of mesocotyl elongation in corn seedlings by applying auxin paste to the cut surfaces of coleoptiles from which the tips were excised. Goodwin (10) suggested that the inhibition must be more complex, proposing an effect of light on cell division as well as cell elongation; and Schneider (23) obtained some light inhibition of isolated mesocotyl sections. Mer (18) also failed to obtain evidence in support of the hypothessis, although he used white light and exposures of up to a week so the experiments are hardly comparable. Galston and Hand (9), working with etiolated peas stem sections, proposed that the inhibition of elongation did not involve auxin at all.

A combination of primitive light sources and optical filters clearly limited the early work. With seedlings of dicots, both blue and red light may inhibit hypocotyl elongation (4, 17, 27) and the responses may be quite different and easily separable either in time (17) or by site of perception (4). Inhibition of mesocotyl elongation in grass seedlings, however, has long been known to be a response to red light only (3, 10, 12, 29, 30).

As part of a general characterization of light-inhibited elongation, we performed experiments which tested van Overbeek's theory of light-inhibited growth. Certain artifactual results of past work were avoided by using excised segments which included just the coleoptile and the apical cm of mesocotyl (or parts therefrom), by taking into account the fact that auxin-producing centers can be regenerated in decapitated coleoptiles (5, 8, 25) and the recognizing that auxin uptake can be greatly reduced if there is no cut surface, apical to the elongating cells, to act as a port of entry (1). We have concluded that light-inhibited elongation can be antidoted by exogenous auxin, consistent with the theory as originally presented by van Overbeek.

MATERIALS AND METHODS

Corn seeds (*Zea mays* L. WF9 × Bear 38, Bear Hybrid Seeds, Decatur, Ill.) were sown dry on two layers of water-saturated absorbant paper (Kimberly-Clarke, supercrepe Kimpack). Growth was in complete darkness at 30 C and 80% relative humidity. At age 72 hr segments which included the coleoptile (or parts therefrom) and the apical cm of mesocotyl were excised in dim green light (500–560 nm, 530 maximum) and stored for not more than 1 hr in 1% sucrose on ice. Segments were floated in open Petri dishes on 10 mM KH_2PO_4 (pH 6) containing 30 mM sucrose, 77 μM chloramphenicol, and various concentrations of IAA. The mesocotyl portion of the segment was measured to the nearest 0.5 mm after 16 hr at 30 C in darkness or under red light. Irradiation with fluorescent tubes (Sylvania warm white) passed through red plastic (Rohm and Haas, No. 2423) gave approximately 1,750 ergs cm^{-2} sec^{-1} at tissue level (590–730 nm, maximum −630 nm). When intact seedlings were grown in the light (Fig. 1) the identical light source was used with from 1,600 to 1,800 ergs cm^{-2} sec^{-1} at seed level. The mesocotyl was measured at 24-hr intervals.

RESULTS AND DISCUSSION

The mesocotyl of intact plants elongated rapidly when the corn seedlings were grown in the dark, but hardly at all when grown in red light. When seedlings were transferred from darkness to red light at 3 or 4 days the mesocotyl elongation rate decreased almost to zero (Fig. 1). This inhibition of mesocotyl elongation by red light also could be demonstrated in excised tissue (Fig. 2).

The experiment presented in Figure 2 leads to several conclusions. (*a*) Elongation of the 1-cm mesocotyl segment (intact coleoptile attached) was 56% inhibited by light. (*b*) Removing the 3-mm coleoptile tip, the site of auxin supply, did not completely eliminate the dark elongation increment. It is well known (5, 8, 25) that an actively exporting auxin supply site can be regenerated in decapitated coleoptiles; this regeneration process (or perhaps auxin export by, or transport from, the newly regenerated auxin supply site) may also be inhibited by light. (*c*) Even in the

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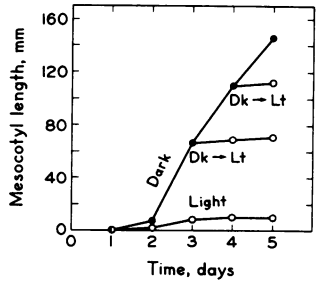

FIG. 1. Red light-inhibited mesocotyl elongation in intact seedlings, time course. Seedlings were grown at 30 C and 80% relative humidity. Fluorescent irradiation (Sylvania warm white) through Rohm and Haas Plexiglas (red No. 2423) gave 1,740 ergs cm^{-2} sec^{-1} at seedling level.

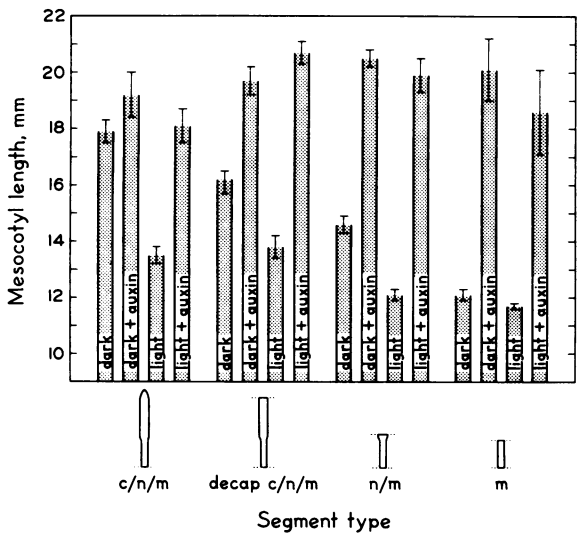

FIG. 2. Effect of attached coleoptile, attached decapitated coleoptile, and attached coleoptile/mesocotyl node, and of red light and auxin on mesocotyl elongation. The excised apical cm of the mesocotyl was grown for 16 hr in buffered sucrose by itself (m), or with the mesocotyl/coleoptile node attached (n/m), or with the node and decapitated coleoptile attached (decap c/n/m), or with the node and intact coleoptile attached (c/n/m). The apical 3 mm of the coleoptile was removed from the decapitated segments. Light, supplied by fluorescent lamps (Sylvania warm white), was passed through red plastic (Rohm and Haas red Plexiglas No. 2423) to give 1,740 ergs · cm^{-2} sec^{-1} at tissue level. The auxin concentration was 57 μM. Data are given as the mean ± the SE.

mesocotyl alone, or if just the basal 1 to 2 mm of the coleoptile and the node remained attached to the 1-cm mesocotyl segment, there evidently was still some regeneration of an auxin supply system. The appearance of this new auxin supply system again was inhibited by light (see further discussion below, Table I). (*d*) Exogenously supplied auxin reversed light inhibition in all cases.

The concentration dependence for auxin reversal of the light inhibition of growth is shown in Figure 3. Auxin increased mesocotyl elongation of the dark-grown segment somewhat (intact coleoptile attached), but the increase was essentially matched in the auxin-treated light-grown segments (Fig. 3A). Significantly elongation of the excised mesocotyl segment alone (node and coleoptile detached) was not inhibited by light at any of the auxin concentrations tested (Fig. 3B).

Anker (1) has clearly demonstrated that the cuticle represents a formidable barrier to auxin uptake. Testing increasing auxin concentrations was therefore especially necessary when the intact coleoptile was left attached to the 1-cm mesocotyl segment, *i.e.* when there was no apical cut surface to serve as a port of entry for auxin. Figure 3 shows that the auxin concentration which is optimal for elongation of isolated mesocotyl segments (Fig. 3B) is clearly suboptimal for mesocotyl growth when the node and intact coleoptile remain attached.

As described above, even elongation of the excised mesocotyl segment was always slightly, and occasionally more than slightly (Table I), inhibited by light. Whenever results such as those in Table I were attained the endogenous elongation of these 1-cm dark-grown segments was usually high, *e.g.* 4 to 5 mm, rather than the usual 1 to 3 mm. It is conceivable that these excised segments can sometimes initiate auxin synthesis, perhaps in a way similar to regeneration of the auxin supply center in the coleoptile. In fact, Evans and Schmitt (8) suggested that a coleoptile segment which had come from a repeatedly decapitated coleoptile was capable of initiating auxin biosynthesis. Similarly, Stahl (24) found that a soybean hypocotyl segment, excised from its endogenous auxin supply, would quickly decrease its elongation rate to a low endogenous level; however, after 3 to 4 hr the elongation rate of the segment would increase slowly, eventually reaching a rate almost equal to that attained if auxin had been added to the medium.

Bertsch and Hillman (2) reported that red light would inhibit elongation of pea stem sections if sucrose were present in the medium. This result was not obtained with corn mesocotyl sections (Fig. 2); nor was sucrose capable of reversing light-inhibited mesocotyl elongation in the "c/n/m" segments (Table II). However, there is a possible explanation for the results of Bertsch and Hillman (2). It is possible, first, that excised elongating segments, under some circumstances, may be capable of regenerating auxin-supplying cells, and, second, that light may inhibit this regenera-

Table I. Light-inhibited Elongation of the Apical 1 cm Mesocotyl Segment

The excised apical 1 cm mesocotyl segment (coleoptile and node detached) was grown for 16 hr in buffered sucrose ± 57 µM IAA and ± red light (see Fig. 2). The experiment shows that occasionally results unlike those of Fig. 2 are obtained, i.e. the mesocotyl segment grows, the growth increment is suppressed by red light, and auxin reverses the suppression.

Auxin	Mesocotyl Length	
	Dark	Light
	mm	*mm*
No	14.7 ± 0.8	11.9 ± 0.3
Yes	19.7 ± 1.1	18.9 ± 1.0

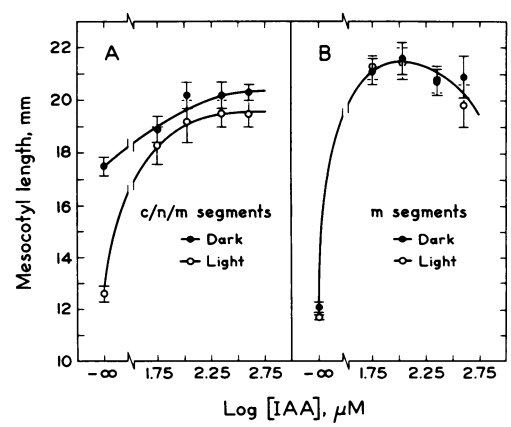

FIG. 3. Auxin reversal of light-inhibited elongation. The excised apical cm of mesocotyl was incubated for 16 hr in buffered sucrose by itself (m segment) or with the intact coleoptile attached (c/n/m) segment) in the presence of increasing auxin concentrations. Red light was given at 1740 ergs · cm^{-2} per sec (see Fig. 1).

Table II. Sucrose does not reverse light-inhibited mesocotyl elongation.

Segments (from 72 hr dark-grown corn seedlings) consisting of the apical cm of the mesocotyl) and attached intact coleoptile were incubated, ± IAA and various sucrose concentrations, at 30 C in 10 mM KH_2PO_4, pH 6.0, containing 77 μM chloramphenicol. Light-treated samples had continuous red irradiation at 1680 ergs cm^{-2} sec^{-1} as described in Materials and Methods. Elongation is expressed as the increase in length of the apical 1 cm mesocotyl segment ± the standard error.

Treatment	Elongation
	mm
Dark	5.9 ± 0.5
Light	2.4 ± 0.3
Light + 50 μM IAA	7.2 ± 0.2
Light + 1 mM sucrose	3.1 ± 0.3
Light + 3 mM sucrose	3.0 ± 0.3
Light + 10 mM sucrose	2.5 ± 0.4
Light + 30 mM sucrose	3.7 ± 0.5
Light + 100 mM sucrose	2.6 ± 0.5

tion process (or perhaps auxin export by, or transport from, the newly regenerated auxin supply site). It may be that this regeneration process is energy-limited for the pea stem segments of Bertsch and Hillman, but not for the corn mesocotyl segments as grown for the experiments described herein. Hence, the sucrose-stimulated growth observed by Bertsch and Hillman would be caused by a regenerated endogenous auxin supply system. Growth due to this cause may be susceptible to inhibition by light, as we have noted above.

It also has been reported that GA can substitute for exogenous IAA in elongating systems which contain both auxin-producing cells and auxin-responsive cells (see 19, 22). Apparently, GA increased the amount of diffusible auxin (22) by enhancing IAA synthesis (15). These conclusions of Muir *et al.* (15, 19, 22) were supported by experiments with corn (Table III). GA reversed light-inhibited elongation at 10 μM. Furthermore, GA had no significant effect on excised elongating cells, indicating that GA probably acted on the auxin-synthesizing cells of the coleoptile when it enhanced mesocotyl elongation in the "c/n/m" segments (Table III), though some entirely independent pathway cannot be eliminated as a possibility.

CONCLUSIONS

The auxin hypothesis of light-inhibited elongation (11, 28, 30) was not supported by subsequent studies (9, 10, 18, 23). However, when excised segments which included only the involved cells (auxin-supplying, auxin-transporting, auxin-responsive) were used, and when data interpretation took into account the facts that auxin supply centers can be regenerated (5, 8, 25) and auxin uptake can be significantly inhibited by the cuticle (1), then one conclusion was inescapable. Light somehow prevented the arrival of auxin at the responsive mesocotyl cells. The hypothesis as originally presented by van Overbeek (28) was supported.

Light could inhibit auxin arrival at the mesocotyl cells by inhibiting export by the supplying cells. An inhibition of auxin synthesis could inhibit auxin export by producing cells; an inhibitory effect of red light on IAA synthesis has been characterized by Muir and Chang (19). Such a possibility was favored by van Overbeek (28) since excised coleoptile tips, previously exposed to light, exported less auxin into agar blocks than the dark controls. While Schneider (23) arrived at an opposite conclusion after similar experiments, later experiments with corn led to observations that agreed with those of van Overbeek, *i.e.* 5-mm corn

Table III. GA reversal of light-inhibited mesocotyl elongation.

Segments were incubated for 18 hr in 10 mm KH_2PO_4, pH 6.0, containing 30 mM sucrose and 77 μM chloramphenicol. Data are presented ± the standard deviation

Treatment	Elongation	
	1 cm mesocotyl segments coleoptile intact	1 cm mesocotyl segments coleoptile detached
	mm	*mm*
Dark	7.1 ± 2.5	2.7 ± 0.9
Light	3.0 ± 1.3	1.2 ± 0.9
Light + 10 μM IAA	7.5 ± 2.0	9.1 ± 2.4
Light + 10 μM GA	7.0 ± 2.0	4.3 ± 1.3

coleoptile tips from red illuminated plants produced just over half as much auxin as tips from dark control plants (6). The light-induced 50% decrease in auxin export from corn coleoptile tips, as measured in short term experiments (6), would appear insufficient to account for the almost complete cessation of growth induced by red light and illustrated in Figure 1. However, experiments to be published elsewhere indicate that shortly following the onset of illumination the mesocotyl growth rate declines, reaching a rate which is about 50% of its dark rate by 2 hr. This new rate is maintained until about 6 hr after illumination, when the rate begins decreasing again (provided the illumination has been continuous) and by 8 hr it has almost reached zero. It seems likely that the red light-induced decrease in auxin export could account for the first decline in growth rate, but at present, there is no evidence in support of any particular mechanism for the second.

Light might also inhibit auxin arrival at the mesocotyl cells by affecting auxin transport (13, 14, 20, 26 and references therein). A review of the literature makes it clear, however, that such an inhibitory effect of light has not been established.

The involvement of phytochrome in red light-inhibited mesocotyl elongation has not yet been definitively demonstrated. While the concentration of the far red-absorbing form of phytochrome immediately after irradiation seemed to correlate with subsequent inhibition of mesocotyl elongation (16), far red light was not capable of fully reversing red light-inhibited elongation (3, 16). Furthermore, the action spectrum for the inhibition of mesocotyl elongation does not clearly point to phytochrome as the sole receptor pigment (29; Vanderhoef and Briggs, unpublished results).

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