Zea Shoot Development in Response to Red Light Interruption of the Dark-Growth Period. I. Inhibition of First Internode Elongation

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Abstract. Brief, low energy (approximately 400 Kerg cm⁻²) red light interruption of the early dark-growth period of Zea mays L. cv F-M Cross induced inhibition of first internode elongation which was maximal whenever light interruption occurred from 2.5 to 4 days of seedling age. Two-thirds of the maximal inhibition occurred in the tissues constituting the region 0 to 2 mm below the coleoptilar node at time of light treatment. The coleoptile tip showed the greatest sensitivity for red light reception in the internode response. Far-red light exposures following red light treatments reversed the red light effect. However, far-red light alone inhibited first internode elongation as effectively as red light of similar dosage.

Low intensity red irradiation (600–700 nm) has been routinely employed in investigations of the physiology of etiolated grass seedlings as a pretreatment for more uniform shoot development and frequently as a manipulation light source (15). A developmental effect of red light under such conditions is an inhibition of first internode elongation. Inhibition of Avena first internode elongation by low intensity red light exposures is nearly complete (2, 3), but the response in Zea and other grasses is generally only partial, even with continuous red light exposures (14). The growth responsive and photoreceptive regions of etiolated Avena shoots in the red-light-induced inhibition response have been investigated (2, 10, 12), but comparable studies of etiolated shoots of Zea have not been reported.

This report presents results of our studies of Zea first internode elongation in response to red light interruption of the dark-growth, including the response to red and far-red light. Photoreceptor and developmental response regions of the shoot have been identified.

Materials and Methods

Experimental Procedure. Seeds of Zea mays L. cv F-M Cross (Ferry-Morse Seed Company) were surface sterilized in 0.2 % sodium hypochlorite (w/w), washed in running deionized tap water, then sown embryo side up on wet paper tow on pads in covered plastic containers. After 40 hr development in the dark, uniformly developed seedlings were selected and transferred to 0.67 % agar in 14 × 2 cm petri dishes under dim green illumination. Subsequent development of the seedlings, except during the brief red or far-red light exposures, took place in the dark at 25 ± 1° and 80 to 100 % relative humidity. Under these culture conditions the logarithmic phase of first internode elongation persisted from about 2 days until about 6 days of age (time after imbibition). Routinely, seedlings were harvested at 6 days of age and the excised internodes were measured to the nearest millimeter. For the controlled exposures to red or far-red light, samples of 10 to 20 seedlings each were randomly selected and irradiated outside the controlled environment of the dark germination cabinet, then returned for further dark development. Control samples were manipulated similarly, including brief green light exposures, except that they received no other light treatment.

Light Sources. Transmission characteristics for the 3 light systems employed are shown in Fig. 1. The dim green manipulation light was a 22 watt cool white fluorescent lamp covered with 2 sheets of green and 1 sheet of amber filter mediums (Cinemoid Nos. 39 and 33). (All Cinemoid filters were obtained from Century Lighting, Incorporated, 3 Entin Road, Clifton, New Jersey 07014.) This light source, at the usual working distance of 100 cm, induced no measurable effects on internode elongation when exposures were less than 15 min. Light from a 500 watt incandescent reflector flood lamp...
filtered through 5 cm of water, then through appropriate combinations of liquid and solid filters was used for the red and far-red light sources. The red spectral region was isolated by 3 cm of 0.1 m CuSO₄ contained in a sealed plexiglass cell and a solid filter composed of 2 sheets of red and 1 of amber filter mediums (Cinemoid Nos. 6 and 33). Far-red light was obtained by using a 3 cm liquid filter consisting of 1 Kg FeSO₄(NH₄)₂SO₄·6H₂O dissolved in 3 liters of 0.5 N H₂SO₄ and a solid filter composed of 2 sheets of Cinemoid No. 6, one of Cinemoid No. 5A, and 9 sheets of blue cellophane.

Seedlings were irradiated from above, except for 1 set of experiments where irradiation was unilateral. The internode elongation response could be saturated with red light (100-1000 Kerg cm⁻²) with exposures of 5 min or less. Intensities were measured with a Photovolt Electronic Photometer, Model 501-M, using phototube E calibrated to erg cm⁻² sec⁻¹ with a YSI Model 65 Radiometer. Calibration of the latter is ultimately traceable to a standard lamp.

Statistical Interpretation. Standard errors of the means (S.E.) and t-tests for significant differences between paired treatment and control means were calculated by methods presented in Snedecor (13).

Results

Red Light Response vs. Age at Treatment. Red light interruption of the dark-growth period of Zea seedlings induced the same degree of inhibition regardless of seedling age at time of interruption from 2.5 to 4 days (table I). This developmental period corresponded to the early logarithmic elongation phase of the first internode.

Localization of Growth Response to Red Light. Preliminary experiments using India ink markings on the internode had indicated that most of the elongation of etiolated internodes, during seedling development from 2.5 to 6 days of age, occurred in the apical tissues of this organ. Results of a subsequent experiment are presented in table II. In this particular experiment duplicate samples of seedlings selected for uniform shoot development were irradiated with 400 Kerg cm⁻² red light, then the internodes were marked, along with those of un-exposed control seedlings, with India ink 1 mm and 2 mm below the coleoptilar node. The greatest increase in length of the control internodes during the subsequent dark-growth period occurred in the region composing the apical 0 to 2 mm of this organ at the time of marking, while 67% of the total internode inhibition in the red light exposed seedlings occurred in this same region.

Table III. Effects of Brief Irradiation of Different Regions of 66-Hr-old Zea Shoots With Red Light Sufficient to Saturate the Internode Elongation Response

Table I. Elongation Response of Zea First Internodes to Red Light Interruption (400 Kerg cm⁻²) of the Dark-growth at Various Developmental Ages

<table>
<thead>
<tr>
<th>Age at interruption (days)</th>
<th>Internode mean length (mm ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>41.0 ± 2.6</td>
</tr>
<tr>
<td>3.0</td>
<td>41.4 ± 2.2</td>
</tr>
<tr>
<td>3.5</td>
<td>41.8 ± 2.1</td>
</tr>
<tr>
<td>4.0</td>
<td>40.6 ± 2.1</td>
</tr>
<tr>
<td>Dark control</td>
<td>48.7 ± 2.4</td>
</tr>
</tbody>
</table>

1 All treatment means significantly different from control mean at P = 0.05.

Table II. Elongation of Different Regions of Zea First Internodes in Response to Brief Red-light Interruption of the Dark-growth Period at 66 Hrs of Age

<table>
<thead>
<tr>
<th>Internode region</th>
<th>Control internode</th>
<th>Red treated internode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (mm ± S.E.)</td>
<td>% of Total elongation</td>
</tr>
<tr>
<td>Apical 0 - 1 mm</td>
<td>34.2 ± 2.9</td>
<td>66</td>
</tr>
<tr>
<td>Apical 1 - 2 mm</td>
<td>7.9 ± 1.0</td>
<td>14</td>
</tr>
<tr>
<td>Basal 2 - 10 mm</td>
<td>17.9 ± 1.2</td>
<td>20</td>
</tr>
</tbody>
</table>

1 Mean length significantly different from that of corresponding control section at P = 0.05.
Table IV. Effects of Far-red Light Exposures on Dark-grown and Red Light Exposed Zea Seedlings

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Internode mean length (mm ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark control</td>
<td>57.7 ± 3.3</td>
</tr>
<tr>
<td>Red</td>
<td>45.3 ± 3.8</td>
</tr>
<tr>
<td>Far-red</td>
<td>45.0 ± 2.5</td>
</tr>
<tr>
<td>Red/Far-red</td>
<td>52.6 ± 3.4</td>
</tr>
</tbody>
</table>

1. Treatment means significantly different from control mean at P = 0.05.

coleoptile tip was the most sensitive site of photoreception, while the tissues in which most of the growth response occurred were only partially responsive to the red irradiation.

**Effects of Far-red Light Exposures.** Involvement of a phytochrome system in the photoresponse was investigated by irradiation of seedling samples with red (400 Kerg cm\(^{-2}\)) and far-red (500 Kerg cm\(^{-2}\)) light. Each exposure was of 5 min duration and, in the case of red followed by far-red, the interval between exposures was always less than 5 min. Results of a typical experiment (table IV) show that the red response could be reversed by a subsequent exposure to far-red of similar dosage. However, the same far-red dosage which reversed the red effect, when applied alone, induced inhibition to the same extent as the red light exposure.

**Discussion**

Results of investigations reported here show that the red-light-induced inhibition of Zea first internode elongation is localized in the apical region of this organ (table II). This region responds maximally at an early age and maintains a constant responsiveness well into the logarithmic phase of internode elongation (table I). Since the Zea first internode has a persistent meristem located immediately below the coleoptilar node (1), our data suggest that cell enlargement and/or cell division were affected by red irradiation. However, the anatomical studies confirming this interpretation have not been done as reported for Avena (2, 10).

The photoreceptor region for red-light-induced Zea internode inhibition need not correspond to the tissue region in which the response occurs (table III). A similar phenomenon has been observed in Avena, but no relative comparisons of effectiveness between photoreceptor regions were made (10). Our data indicate that the coleoptile tip is the most effective receptor for the red-light-induced response of the internode. The limited internode inhibition in response to a red light interruption might be accounted for by a red-light-induced interruption of auxin flow, if there were direct dependence on auxin for internode growth as suggested for Avena (10, 11). Such interpretation is further suggested by evidence that red light suppresses the diffusion of auxin from excised Zea coleoptile tips, a response which decays during the dark period following red irradiation (4).

Reversal of the red light effect by subsequent far-red irradiation indicates mediation of the internode elongation response by a phytochrome system (table IV). The involvement of a phytochrome photoreceptor in developmental responses can be inferred from reports of phytochrome localization in the Zea shoot (5, 6, 7). Briggs and Chon (5, tables I and II), in their study of the red light effect on Zea phototropic responses, have shown that the distribution of red light sensitivity in the Zea shoot correlates with relative phytochrome distribution. Our results (table III, column 3) show a similar distribution of red light response. However, far-red exposures of the same dosage that reversed the red light effects, when applied alone, induced an inhibition equal to the red light inhibition. Evidence that prolonged, low intensity far-red irradiation can cause the same effects as red light in Zea shoot development by maintaining a low, active, photostationary level of far-red absorbing phytochrome has been reported (8, 9). That such a photostationary level was established during our far-red irradiation experiments seems unlikely since the effects of the far-red light are complete, but different depending on the presence or absence of prior red light exposure. Neither can our data be explained by the presence of a low energy, far-red-irreversible red light response which has been described for Avena (3, 10). The complete reversal of the red effect by subsequent far-red exposures in our experiments preclude such an interpretation.

In conclusion, our results support the interpretation that the red-light-induced internode inhibition of dark-grown Zea shoots is mediated by a phytochrome system in the coleoptile tip. The physically separated sites of photoreception and growth response in the shoot suggests this organism is potentially useful for studies of phytochrome-mediated control of early growth of the grass shoot.

**Literature Cited**