Altering the Axial Light Gradient Affects Photomorphogenesis in Emerging Seedlings of Zea mays L. 

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ABSTRACT

The axial (longitudinal) red-light gradient (632 nanometers) of 4 day old dark-grown maize seedlings is increased by staining the peripheral cells of the coleoptile. The magnitude of increase in the light gradient is dependent solely on the light-absorbing qualities of the stain used. Metanil yellow has no effect on the axial red-light gradient, while methylene blue causes a large increase in this light gradient. These stains did not affect growth in darkness or the sensitivity of mesocotyl elongation to red light. However, mesocotyl elongation was altered for the dark-grown seedlings stained with methylene blue when these seedlings were transplanted, covered with soil, and permitted to emerge under natural lighting conditions. These observations are consistent with the idea that there is a single perceptive site below the coleoptilar node, and suggest that this perceptive site receives the actinic light which has traveled downward through the length of the shoot from an entry point in the plant tip region.

Light-inhibited elongation of cereal seedling mesocotyls has been well studied as a model system for plant photomorphogenesis. It is known that red light initiates the inhibition of mesocotyl elongation (6, 10), and that the pigment responsible for this action is phytochrome (3, 5, 12). However, the mechanism of pigment action and the later steps in the transduction sequence are still not defined completely. This has been due in part to confusion concerning the site(s) of photoperception for the measured response.

Initial studies demonstrated that mesocotyl inhibition could be affected by irradiation of either the region just below the coleoptilar node (1, 6), or the coleoptile tip (4, 6). Early and recent evidence suggested that light inhibits mesocotyl growth by limiting the supply of auxin from the coleoptile (7, 25, 27). Thus, it seemed reasonable that a photoperceptive site for mesocotyl inhibition could be located distally in the coleoptile. It was still suggested, however, that light received by the coleoptile was actually transmitted downward within the shoot to a perceptive site some distance away from the region where light was incident on the shoot (6, 18).

The role of tissue optical qualities in growth and developmental responses has long been recognized. Phototropism, for example, results from a spatially differential growth response based on a light gradient developed across the shoot tissue (2, 20, 21). Optically dependent models for photomorphogenesis also have been proposed (11). For example, the extreme light sensitivity of mesocotyl tissue (13), and the axial light transmitting qualities of cereal seedling tissue (14, 15, 19), together support the contention that light perception occurs solely within the mesocotyl. A correlative relationship between red-light-initiated photomorphogenesis and the optical qualities of the shoot tissue provided further evidence in support of this contention (15). Studies by our group have also demonstrated that light incident on the coleoptile could affect photoconversion of phycocyanin within the mesocotyl tissue (19). Combined, these data strongly favor the existence of a single light perceptive site within the mesocotyl tissue. However, no experiments have shown this directly under natural growing conditions.

To demonstrate that axially transmitted light significantly affects morphogenesis in the nonemergent mesocotyl, tests were conducted under growing conditions in which shoots emerged from soil under a natural light regime. Under the fluence and spectral distributions used in previous investigations (15), it remains possible that light-induced responses in other regions of the seedling had not been activated. Experiments under natural growing conditions permitted a complete assessment of the possible factors which could regulate mesocotyl photomorphogenesis.

This present work describes a method for selectively altering the axial light gradient of etiolated corn seedling shoots. Seedlings with altered optical qualities have been used to examine the role of axial light transmittance in the morphogenesis of the buried mesocotyl.

MATERIALS AND METHODS

Plant Material. Hybrid corn (WF9 x Bear 38, Custom Farm Seed Res., Decatur, IL) was used for all experiments. After overnight imbibition in distilled H2O, seeds were planted according to the type of experimental assay employed: (a) for spectrophotometric analyses, seeds were allowed to germinate and grow in darkness for 4 d at 24.0 ± 0.5°C in covered plastic trays (about 40 seeds/tray) on Kimpak (Kimberly-Clarke, Neenah, WI) soaked with distilled H2O; (b) for growth rate measurements, imbibed seeds were individually sown about 2 cm deep in glass seed vials (9 x 2 cm i.d.) filled with vermiculite and moistened with distilled H2O. Individual vials were then placed in a dark humid chamber (24.0 ± 0.5°C) for 3 d; (c) for photomorphogenesis experiments, presoaked seeds were evenly sown in plastic trays half-filled with vermiculite and soaked with distilled H2O. Trays containing planted seeds were covered with plastic wrap and placed in a dark room for 3 d at 24.0 ± 0.5°C. On the 3rd d after planting, selected seedlings were treated and transplanted as described below (see Photomorphogenesis in “Materials and Methods”).

Spectrophotometry. All assessments of the light-attenuating qualities of corn seedlings were performed as described previously.
In brief, axial light attenuation was measured at 632 nm along single etiolated corn seedlings using localized phytochrome conversion as an *in situ* quantum counter. Phytochrome conversion was assayed across each seedling 1 mm below the coleoptilar node. This photoconversion was accomplished by an actinic irradiation incident at a point on the coleoptile some distance from the region of spectral measurement. Changing this distance between the point of actinic irradiation and the position of spectral measurement altered the amount of phytochrome photoconverted. These measurements, and a dose-response curve for phytochrome photoconversion in this system, were used to calculate the number of quanta transmitted along a given length of shoot tissue (coleoptile plus inner leaf) (19).

Photoconversion could also be induced by stray actinic quanta. The amount of this spurious photoconversion was measured by wrapping the coleoptilar region with black tape to eliminate the direct (nonstray) irradiation and then proceeding with the attenuation measurements induced only by the stray light. In this manner, photoconversion measurements made without the black tape were corrected for the stray light effects on phytochrome photoconversion.

Absorption spectra of specific tissues above the coleoptilar node were measured transversely across a seedling at a given region along a single shoot as described above and in detail previously (19). For these measurements, a rolled white tissue (absorbent paper) served as a reference.

Seedlings used for axial attenuation and absorption measurements often required tissue preparation and selective staining of the coleoptile. Under dim green light, the entire coleoptile, from the node to the shoot apex, of 4 d old dark-grown seedlings was prepared for staining by lightly abrading this area with a cotton applicator impregnated with wetted carborundum powder. After abrasion, seedlings were rinsed with distilled H2O and immersed in one of two staining solutions (0.05% methylene blue: \( A_{\text{max}} = 663 \text{ nm} \); 0.1% metanil yellow: \( A_{\text{max}} = 434 \text{ nm} \)) for 1 or 4 min. Stained plant tissues were blotted with paper tissue and lightly coated with a thin film of silicone grease to prevent evaporative water loss from the abraded and stained area during the spectral measurements necessary for the assay of light attenuation. Abraded controls were prepared as described above with the omission of the staining step. To obtain a seedling with a partially stained coleoptile, only the lower portion (about 4 mm) of the coleoptile was abraded and then stained for 4 min. The remaining unabraded cutinous layer enveloping the coleoptilar tip was impermeable to the tissue stains.

**Growth Rate Measurements.** Three d old dark-grown seedlings

![Absorption spectra](https://www.plantphysiol.org/)

**Fig. 1.** Cross-sectional absorbance of coleoptile and leaf tissue. Absorption measurements were recorded across individual coleoptile or inner leaf tissues about 0.5 cm below the coleoptilar tip of 4 d old dark-grown seedlings. Where applicable, seedlings were stained for 4 min (see Spectrophotometry in "Materials and Methods"). The measured coleoptilar spectra represent the absorption of light across half of a longitudinally cut coleoptile. Specific mean absorbances listed in the upper right corner of each block are the average values from four separate seedling measurements ± 1 SE. The separate sections of the figure are: A, unabraded coleoptile; B, abraded coleoptile; C, methylene blue-stained coleoptile; D, metanil yellow-stained coleoptile. Individual spectra are identified by: L, leaf; C, coleoptile.
Table 1. Light Transmission Along a Partially Stained Corn Seedling

<table>
<thead>
<tr>
<th>Dose Transmitted (nE)</th>
<th>Metanil yellow</th>
<th>Methylene blue</th>
<th>Abraded control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8 ± 0.2</td>
<td>0.2 ± 0.2</td>
<td>2.9 ± 0.2</td>
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entire shoot length from the seed to the shoot tip and was located 20 cm away from the seedlings. During the growth experiments, the seedlings grew within a 1.5 cm wide clear plastic corridor, which maintained the seedlings in the focal plane of the camera. Data were recorded by mounting the developed negatives and then projecting the images on a screen from which lengths of the mesocotyl and coleoptile were measured and corrected for the actual size.

Photomorphogenesis. Seedlings which had grown for 3 d in total darkness in large covered trays were selected for size (about 3.5 cm seed to shoot tip; about 2.5 cm mesocotyl) and stained, when necessary, for 4 min as before (see Spectrophotometry). Under dim green light, experimental and control seedlings were transplanted into moist vermiculite so that the base of the mesocotyl was located about 4 cm below the top of black plastic pots (9 × 9 × 14 cm). A 1 cm layer of soil consisting of finely sifted peat and loam was used to fill the remaining volume of the pots and completely cover the transplanted seedlings. A clear plastic dome was placed over each individual pot to maintain a high humidity.

Pots containing treated and control seedlings were placed in a greenhouse (about 27°C) for 3 d. Pots were distributed evenly within a tiered rack consisting of three levels separated by metal screens to provide successively lower light intensities. Light was provided by a 1000 W multivapor metal halide lamp (MV 1000/ U; General Electric, Hendersonville, NC) suspended 60 cm above the top growing level of the rack (16 h/d cycle). A distilled H₂O tank was placed between the samples and the light source to absorb excess IR irradiation from the lamp. The entire lamp and rack was shrouded in black cloth to ensure that the samples were only irradiated by diffuse sunlight and direct light from the lamp. This ensured an even balance of irradiation on the experimental pots. Blue and red light filters, when used, consisted of colored Plexiglas (red, No. 2423; blue, No. 2424; Rohm and Haas, Philadelphia, PA) placed underneath the water tank. Measured spectral outputs were: red-λ_max = 620 nm, cutoff below 580 nm; blue-λ_max = 465 nm, cutoff between 540 nm and 760 nm. Plants were lightly watered with distilled H₂O every day. On the 5th d, the individual plastic covers were removed from each pot to permit unhindered leaf elongation. After 6 d, data were recorded by clipping the seedlings at soil level and measuring the distance to the coleoptilar node above or below the point of cutting. All elongation of mesocotyl tissue had occurred before the 6th growing day so that, at the time of measurement, the seedling node was stable at its position (data not shown).

Integrated light intensities were measured with an International Light spectroradiometer (model 700A; International Light Inc., Newburyport, MA).

RESULTS AND DISCUSSION

Axial Light Attenuation. To examine directly the role of a light gradient in light-induced mesocotyl inhibition, one must
change the axial light gradient without significantly perturbing other functions of the seedling. Previous results indicated that the coleoptile was the structure along which light was transmitted particularly for relatively long distances (19). Thus, changing the axial gradient would likely necessitate the alteration of coleoptilar pigmentation.

Alteration of the effective light gradient was accomplished by dyeing the entire coleoptile of young etiolated shoots with tissue stains. Only the red (632 nm) light-attenuating qualities of the treated shoots were measured, since this wavelength is within the primary spectral region of Pr photosensitivity. The photoequilibrium of phytochrome should be constant at about 80% Pfr at this wavelength (22). Only dyes that absorb 632 nm light should affect the magnitude of axially transmitted red light. Neither coleoptilar abrasion nor staining with metanil yellow changed the in vivo light absorption of the coleoptile at 632 nm (Fig. 1, A, B, and D). Thus, as expected, the optical qualities of these treated shoot tissues were not substantially different from previously reported values (Fig. 2) (15, 19). Conversely, coleoptilar staining with methylene blue yielded a significant increase in the red region of both the magnitude of the in vivo absorption spectrum and the magnitude of axial light attenuation (Figs. 1C, and 2).

It was possible to affect the axial light gradient of corn shoots by introducing stains into a short length (about 4 mm) of the coleoptile beginning 1 mm above the coleoptilar node. Although the tissue-transmitted light was incident initially on the unstained apical region, light transmittance still was affected noticeably for blue-stained seedlings (Table 1). This result indicated that the increase in attenuation observed for shoots with coleoptiles which were dyed entirely resulted primarily from a decrease in axial light transmission and not from absorption of light at the point of incidence.

Microscopic analysis of tissue cross sections immediately after staining revealed that the stains were localized mainly within the peripheral cells (about 2 cell layers—data not shown). There was little or no stain present inside the remaining internal cell layers (about 10) of the coleoptile. Moreover, the dye remained concentrated mostly within the originally stained cells for at least 14 h after staining, indicating that internal diffusion of the stains was minimal.

These results permit an analysis of the mode of axial light...
transmission. The effect of specifically localized staining on axial light gradients supported the idea that the internal regions of the shoot contribute minimally to the path of light propagation (14, 16). It also would appear that unstained inner leaf tissues do not provide a primary route for light. The normally high attenuation values for leaf tissue tend to support this conclusion (19). Previous photomorphogenic studies have indicated that light absorption by a leaf can affect a response (11). However, these experiments utilized dicotyledonous seedlings, which are optically dissimilar from corn.

Two possibilities emerge from the observation that seedlings were optically altered as a result of staining the peripheral cells of the coleoptile. Either axial light transmission is limited exclusively to the outer periphery of coleoptile cells, or quanta entering internal coleoptilar regions are scattered to and absorbed within the stained periphery. Although our data can not distinguish between these two possibilities, recent findings from measurements of light gradients across maize shoots have suggested that the coleoptile is a highly light scattering structure at least in the transverse plane, supporting the latter contention (26).

**Growth Rate Measurements.** To test for any extraneous effects of the various tissue treatments on photomorphogenic sensitivity, time course responses were measured for mesocotyl inhibition after direct unilateral red-light irradiation of the whole seedling shoot. This irradiation was not active phototropically (8). Over the fluence range tested, all mesocotyls showed sensitivity to light (Fig. 3). The lower fluence used was just within the saturation range for the low irradiance response for oats (13). If corn responds similarly, then a difference in light sensitivity between tissue treatments would have been reflected in the growth curves. However, this was not seen and thus, the tissue preparations did not appear to alter the light sensitivity of mesocotyl tissue. Under these conditions, the light-promotive response of coleoptiles was different from mesocotyls, responding only at the highest fluence used (Fig. 3). This result provided further evidence that the general light sensitivity of the plant tissues was not affected by tissue manipulations. Red-light stimulation of coleoptile elongation, which was also seen for blue-stained coleoptiles, is expected given previous evidence suggesting a perceptive site for this response within the mesocotyl or the coleoptile base (15). Although only growth rates of the individual seedlings from a single experiment are presented, repetitions revealed that the results of this experiment were typical. The slight differences between individual plants (e.g. inner leaf emergence) within a particular tested fluence range probably reflects a normal variance in the population rather than a specific effect induced by a particular treatment.

**Photomorphogenesis.** All types of seedlings grown under natural lighting conditions in a greenhouse showed an approximately direct linear relationship between light intensity and the degree of mesocotyl inhibition (Fig. 4)(9). Lower light intensities yielded seedlings with longer mesocotyls. If the photoperceptive
site resides within the buried mesocotyl, then a seedling with an altered red-light gradient should respond like a normal plant which had grown under lower light intensity (i.e. the curve would be shifted toward higher fluence rate). To test this, photomorphogenesis was analyzed in seedlings with altered axial red-light gradients. Under white or red light conditions, all plants responded similarly to untreated controls except for methylene blue-stained individuals (Fig. 4, A and B). Under these white and red light regimes, the response of blue-stained seedlings shifted toward lower light sensitivity. Since the axial gradient was affected under these conditions, it follows that this altered response likely resulted from a lower transmitted light fluence at the photosensitive region below the soil surface.

No differences in the response were seen between the treatments under blue-light growing conditions (Fig. 4C). This was expected, given a low blue-absorption for phytochrome, and the lower fluence rates for our blue irradiations. It would appear that the mesocotyls responded to this lower absorbed fluence by elongating above the soil surface. At that point, any effects of an altered axial light gradient could not occur since the mesocotyl tissue was now directly exposed to light. If we had grown seedlings under a higher blue-light intensity, then the response curve for metanil yellow-stained seedlings may have been shifted with respect to the other seedling treatments. This prediction assumes that the blue-light axial gradient is affected in yellow-stained seedlings.

The shift in the dose response (Fig. 4, A and B) seen for blue-stained shoots was probably not a result of absorption of red actinic quanta at the incident surface. An altered response was still seen when the tissue was stained blue in a small section (about 4 mm) just above the node with the apical region left untreated (Fig. 4D). This indicated that apical light reactions, if any, did not affect morphogenic changes within the buried mesocotyl. Additionally, elongation of transplanted seedlings caused a thinning of the stem along the coleoptilar surface (data not shown), so that the absorptive barrier (A467nm) across the coleoptile was lower upon emergence than it was initially (Fig. 2C).

The light attenuating qualities of the soil are much greater than those of the shoot tissue (17, 28). Measurements for our system indicated that light attenuation by soil is about 10 times greater than that of the shoot tissue (data not shown). Therefore, the likelihood that light transmittance through the soil directly and significantly affected mesocotyl elongation without light transmission occurring along the seedling shoot is minimal. Given the magnitude of light attenuation seen for soil, photo-inhibition of mesocotyl elongation via axially transmitted light would also be significant for more shallow planting depths than those used in these experiments.

Went (27) and van Overbeek (25) initially proposed that light affects mesocotyl inhibition by reducing the amount of auxin from the coleoptile. Although at least one study has indicated that a coleoptilar supply of auxin may not be required for the response (23), current literature supports the former proposal (7, 24). It is unlikely that the experimental treatments described here affected the supply of auxin from the coleoptile. If tissue abrasion were to have affected auxin transport, then it most likely would have lowered it. An injury-induced reduction in auxin supply would yield shorter mesocotyls (24), which was not seen. This leaves us with the conclusion that either there is an injury-induced effect on auxin supply which is promoted specifically by methylene blue, which is unlikely, or that light is perceived within the mesocotyl and affects coleoptilar auxin supply through some unknown mechanism.

The present results indicate that the site of photoperception for mesocotyl inhibition resides in tissue which is located beneath the soil surface. This site appears to be photostimulated by light which has reached it mainly via transmission through coleoptilar tissue rather than through the far more opaque soil. Thus, the coleoptile not only provides a protective sheath for the frail primary leaves during emergence, but likely serves as an optical structure through which light can pass and effect photomorphogenesis in plant regions which are not normally exposed to direct solar irradiation.

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