Role of the Cotyledons in the Phototropic Response of *Lavatera cretica* Seedlings

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ABSTRACT
Young seedlings of *Lavatera cretica* L. exhibit positive phototropism. The hypocotyl perceives unilateral illumination with blue light and curves towards the light source by unequal growth. In addition, the cotyledonary lamina perceives the vectorial component of unilateral illumination with blue light and reorients normal to the beam by creating a turgor differential in their pulvini. Excision of one cotyledon resulted in negative geotropic curvature of the hypocotyl, away from the remaining cotyledon. Illumination of the cotyledonary lamina did not participate in the phototropic curvature of the hypocotyl, so long as the lamina was free to reorient to face the beam. When the lamina was continuously exposed to vectorial photoexcitation, elongation of the hypocotyl on the side carrying the cotyledon could be enhanced, or inhibited, depending on the direction of the beam striking its lamina.

Young, de-etiolated dicot seedlings exhibit a positive phototropic response by curvature of their hypocotyls (or stems) towards the source of unilateral illumination. There seems to be disagreement on the question whether this response depends solely on perception of unilateral illumination by the stem itself, or by the cotyledons (or young leaves) as well. On the one hand, Shibaoa and Yamaki (10) have suggested that the leaves participate in the phototropic light perception in sunflower since their excision resulted in partial loss of response. Similarly, Lam and Leopold (6) observed that, in vertical illumination, when one of the sunflower cotyledons was shaded, the hypocotyl curved away from it and, therefore, suggest that the hypocotyl curves in unilateral illumination because of quantitative differences in the light interception by the two cotyledons. Others have concluded that phototropically active light is perceived only by the stem (hypocotyl) itself and that the role of leaves (cotyledons) is restricted to the supply of growth regulators (3, 4, 8, 11). A similar role was suggested for the lamina in the phototropic curvature of the petiole in some other plants (1, 5).

Brennan et al. (2) challenged the interpretation that leaves can participate in directional light perception. Mung bean seedlings exhibited positive phototropism even when their primary leaves were shaded or were unilaterally illuminated perpendicular to their midveins and, thus, intercepted light equally, but not when the stem itself was shaded. Shuttleworth and Black (11) observed that shaded cotyledons did not change significantly the phototropic response of the hypocotyl in cucumber and sunflower seedlings. However, they repeated and confirmed Lam and Leopold’s results but observed that red (not blue) light was responsible for the curvature of the hypocotyl to the side carrying the unshaded cotyledon, whereas only blue light was responsible for the response to unilateral illumination. On the basis of these spectral differences, they concluded that the phototropic response did not involve unequal interception of light by the two cotyledons.

*Lavatera cretica* L. (Malvaceae) is an annual weed growing along roadsides in Israel, whose leaves, as well as its cotyledons, track the sun with great precision throughout their life. As *Malva neglecta* (13) and *Lupinus arizonicus* (12), *L. cretica* leaves possess a pulvinus at the juncture of the palmately veined lamina with the petiole, which adjusts the orientation of the lamina normal to the sun by creating a turgor differential across the appropriate segments of the pulvinus. We have recently shown (9) that the photoreceptor for this response is located in the lamina. The lamina reorients itself normal to a light beam striking it from above even when all its parts are equally illuminated. We have described these as vectorial phototropic responses.

In the present study, we have attempted to contribute to the understanding of the phototropic light perception by de-etiolated dicot seedlings, using *L. cretica* seedlings whose cotyledons contain the photoreceptor by which the lamina is reoriented normal to an oblique light beam.

MATERIALS AND METHODS
Mericarps of *L. cretica* L. were pretreated in 70% (v/v) H2SO4 for 1 h and then thoroughly washed in tap water and planted either in drainered plastic cups (5 cm diameter) or in drained plastic flats containing a 1:1 (v/v) mix of basalt gravel and Vermiculite. The plants were irrigated every 1–2 days with nutrient solution. The plants were grown at 22 ± 1°C in daily cycles of 16 h light/8 h dark. Light was provided from above, by a bank of cool-white WS Gro-Lux fluorescent tubes (Sylvania), providing a photon fluence rate of 150 μE m−2 s−1 (400–700 nm) at plant level. Phototropic illumination was generally provided from a 500-w Quartzline lamp (General Electric) installed in a model No. 10 reflector housing fitted with a hot mirror in front and a cold mirror in the back (Dicrolite Co., Everett, Washington). Photon fluence rate was 150 μE m−2 s−1 (400–700 nm). For hypocotyl illumination, the lamp was placed laterally to the plants with its beam parallel to the cotyledons. For illumination of cotyledonary laminas, the light was directed down at an angle of 45°, in the plane of symmetry of the midveins. A “tip-oriented,” or “base-oriented,” oblique beam is one that is pointed towards the tip or base of the lamina, respectively. In this way, all phototropic responses took place in the plane of symmetry of the midveins. Blue and red light were obtained from the filters described by Lurie (7), at a photon fluence rate of 30 μE m−2 s−1. Photon fluence rates were measured with a Lambda Instrument (Lincoln, Nebraska) model LI-185 Quantum-Meter.

Separate light regimes to the different organs of the seedling shoot were obtained by using traps, consisting of the upper 15 mm of the hypocotyl, attached to a single cotyledon (or both), as follows. Seedlings were dug up and immersed in water. The excision was carried out under water. The hypocotyl then was threaded through a small, lighttight hole in an opaque horizontal...
partition, into a water-filled compartment, which could be darkened, or illuminated, as desired (Fig. 1). Where continuous photostimulation of the cotyledonary lamina was desired, the petiole was also inserted through the hole into the chamber, leaving the lamina flat on the partition. In this condition the lamina is fixed flat on the partition and can be continuously exposed to an oblique (or vertical) beam. Where it was desired to leave the lamina free to reorient, the petiole was left to project above the partition. In either case, the base of the hypocotyl was free to curve, whereas in rooted seedlings, the apical portion of the hypocotyl was free to curve, which results in passive reorientation of the cotyledons. This difference did not affect any of the phototropic responses of the seedlings.

Curvature of the hypocotyl was measured by the angle between the tangents to its basal and apical ends. Orientation of the lamina was measured as its angle of inclination to the horizontal.

Time course of the response in intact seedlings was followed by photographing them repeatedly. Time course of hypocotyl curvature in explants was followed by measurements of successive pencil tracings of the same hypocotyl, made entirely under a green safelight. Final curvature was measured after the response had been completed (6 h).

Results are means (± se) of at least 10 intact seedlings, or 40 explants.

RESULTS

Young intact seedlings, exposed to unilateral illumination, exhibited positive phototropic curvature of their hypocotyls and petioles, as well as vectorial reorientation of their cotyledonary laminas by pulvinar response to face the light source (Fig. 2B).

The responsiveness of the hypocotyl coincided with its growth rate and ceased when elongation had come to an end (Fig. 3). Responsiveness of the cotyledonary lamina remained unchanged from their unfolding to their senescence.

The hypocotyl exhibited approximately the same curvature in response to unilateral illumination when the cotyledons were fully exposed, as when their laminas only were in darkness, or even (for a limited time) after they had been excised (Fig. 4).

In absence of one of the two cotyledons (excised at the node), a vertically illuminated seedling exhibited hypocotyl curvature away from the remaining cotyledon, whether its lamina was covered (Figs. 2D and 5A) or exposed (Figs. 2C and 5B).

In the absence of one of the two cotyledons, a unilaterally illuminated seedling exhibited a positive phototropic response of its hypocotyl (i.e. curvature towards the light source) when its remaining cotyledon was pointing away from the light source (Figs. 2F and 5C), but not when it was pointing towards it (Figs. 2E and 5D). Time course studies of these responses showed (Fig. 6) that when the remaining cotyledon was pointing away from the light source, the curvature of the hypocotyl approached its final value at a steadily decreasing rate, exhibiting a saturation response. When the remaining cotyledon was pointing towards the light source, the hypocotyl initially curved towards the light, but this was subsequently reversed. In decotylized seedlings, the hypocotyl curved towards the light source, also exhibiting a saturation response.

The phototropic curvatures obtained by unilateral illumination of seedlings with a single cotyledon depended exclusively on the unilateral exposure to light of the hypocotyl itself. When the cotyledonary lamina was covered, the exposed hypocotyl curved towards the light only when the remaining cotyledon was pointing away from the light source, to the same extent as when the lamina was also exposed (compare Fig. 5, G with C and Fig. 5, H with D). When the hypocotyl was shaded, it curved away from the remaining cotyledon, and to the same extent, when the latter was pointing away from the light (Fig. 5E), or towards it (Fig. 5F). The extent of this curvature was similar to that of vertically illuminated seedlings with a single cotyledon (Fig. 5B).

Using explants with a single cotyledon (Fig. 7), it was found that the darkened hypocotyl curved away from the remaining cotyledon when its lamina was vertically illuminated (B), or in darkness (C), as well as when it was illuminated by a tip-oriented oblique beam (D). When, however, it was illuminated by a base-oriented oblique beam, the curvature of the hypocotyl was reversed, i.e. towards the remaining cotyledon (E). The cotyledonary pulvinus exhibited the typical vectorial phototropic responses when its lamina was exposed to an oblique beam (D and E).

However, base-oriented illumination of the lamina failed to reverse the curvature of the hypocotyl (towards the remaining cotyledon) when the petiole was allowed to project above the partition of the explant chamber (cf. F and E). This was due to exposure of the petiole to light because it took place only when the lamina was left free to orient normal to the beam, not when it was held horizontally and thus maintained at the same oblique angle to the base-oriented beam. Figure 8 quantifies the effects that tip-oriented and base-oriented illumination of the lamina have on curvature of the hypocotyl, when the lamina is free to reorient normal to the beam and when it is not free to do so. The time course of curvature of the hypocotyl away from the remaining cotyledon was greatly accelerated, and to the same extent, when the lamina was exposed to a vertical, or a tip-oriented beam, as compared to darkness (Fig. 9).

It was further found that, when the hypocotyl of the explant inside the chamber was not in darkness, but unilaterally illuminated (Fig. 10), it exhibited considerable positive phototropic curvature (i.e. towards the light source), whether the lamina of the remaining cotyledon was in darkness (C and D), or illuminated with a base-oriented oblique beam (compare A and E), or a tip-oriented oblique beam (compare B and F). However, the magnitude of curvature was greater when the remaining cotyledon was situated on the nonilluminated side of the hypocotyl (B, D, and E) than on the illuminated side (A, C, and F). When the remaining cotyledon was situated on the nonilluminated side, curvature of the hypocotyl was hardly affected by the direction of the beam incident on the lamina or by its total absence (B = D = E). When the remaining cotyledon was situated on the illuminated side, curvature of the hypocotyl was the same whether the lamina was
Fig. 2. Phototropic responses of 3- to 4-day-old seedlings of *L. cretica*, with both cotyledons intact (A, B), or after excision of one cotyledon (C-F). Arrows indicate direction of light beam. E and F, remaining cotyledons pointed towards light source (E) or away from it (F). Exposure, 6 h (× 1.6).

Fig. 3. Changes in the mean daily rate of elongation (∆) and in phototropic responsiveness (○) expressed as maximal curvature after 6 h of unilateral illumination of *L. cretica* seedlings hypocotyl.

Fig. 4. Phototropic response of the hypocotyl of 3- to 4-day-old seedlings of *L. cretica* to unilateral illumination (6 h), as affected by shading the cotyledonary laminas, or their excision.

in darkness or exposed to a tip-oriented beam but was considerably greater when it was exposed to a base-oriented beam (A > C = F).

In the following experiments, explants were used from which only the radicle had been excised. When the hypocotyl was unilaterally illuminated, its curvature was the same whether the laminas of the two cotyledons were in darkness or exposed to an oblique beam (Fig. 11). When the hypocotyl itself was in darkness, it exhibited positive phototropic curvature in response to oblique illumination of the cotyledonary laminas, but to a lesser degree than when it was itself unilaterally illuminated. However, when the laminas were free to reorient to a position normal to the beam (by allowing their petioles to project above the explant chamber), the hypocotyl failed to show any curvature.

The effects of base-oriented illumination of the lamina on reversing the curvature of the darkened hypocotyl took place in blue light but not in red light. Unilateral illumination of the hypocotyl itself with red light failed to produce a positive phototropic curvature when the lamina was illuminated with blue light, either base-oriented or tip-oriented (Fig. 12).

Fig. 5. Hypocotyl curvature in 3- to 4-day-old seedlings of *L. cretica*, after excision of one cotyledon, as affected by unilateral illumination (6 h) when the cotyledonary lamina (A, G, H) or the hypocotyl itself (E, F) are shaded. Arrows indicate direction of light beam.

DISCUSSION

The overall phototropic response of *L. cretica* seedlings to unilateral illumination combines curvature of the hypocotyl and the cotyledonary petioles, as well as reorientation of the laminas.
PHOTOTROPIC RESPONSE OF DICOT SEEDLINGS

FIG. 6. Time course of hypocotyl curvature in unilaterally illuminated, 3- to 4-day-old seedlings of *L. cretica*, after excision of one cotyledon (○ ○, ■ ■) or both (x x).

Fig. 7. Hypocotyl curvature and pulvinar response in explants of 3- to 4-day-old seedlings of *L. cretica*, as affected by the direction of the light beam incident on the lamina of the single remaining cotyledon. Shaded area in dark. Arrows indicate direction of beam. A: initial state of explant; C, controls in darkness; F, lamina free to reorient.

Fig. 8. Hypocotyl curvature in explants of 3-to 4-day-old seedlings of *L. cretica*, as affected by the oblique illumination of the single cotyledonary lamina, when the latter lies flat on the horizontal partition (A, B), or free to reorient (C, D). Shaded area in dark. Arrows indicate direction of beam. (X ~1).

Fig. 9. Time course of hypocotyl curvature (top, away from cotyledon; bottom, towards cotyledon) in the explants shown in Figure 7. B: C: ■ ■; D: ○ ○; and E: ▲▲.

(Fig. 2) The phototropic responsiveness of the hypocotyl to unilateral illumination paralleled its own growth rate and, therefore, may be ascribed to unequal growth on the exposed and shaded sides (Fig. 3).

The responses of *L. cretica* (Fig. 4) are in agreement with those of other dicot seedlings (2, 8, 11). These data support the conclusion that the hypocotyl itself contains the photoreceptor for its positive phototropic curvature in unilateral light since this response did not depend on directional information from the cotyledons, on their being in light or on any immediate supplies from them.

At the same time, the hypocotyl exhibited curvature even in nonunilateral illumination, or in darkness, when one of its cotyledons was excised. The direction of curvature was away from the remaining cotyledons, whether its lamina was in darkness (Figs. 2D and 5A) or in light (Figs. 2C and 5B). This curvature apparently resulted from unequal distribution of factors moving to the
hypocotyl from the cotyledon. This response, therefore, may be described as "negatively organotropic".

The negative organotropic response was modified by interaction with the positive phototropic response. Thus, a unilaterally illuminated hypocotyl curved towards the light, to the same extent, when its single cotyledon, covered or exposed, was pointing away from the light (Fig. 5, C and G), because these opposite responses were acting on the opposite sides of the hypocotyl. The hypocotyl failed to curve, to the same extent, when the single cotyledon, covered or exposed, was pointing towards the light (Figs. 5D and 10H) because the negative organotropic response was antagonized by the positive phototropic response acting on the same side of

the hypocotyl. Time course studies (Fig. 6) indicated that the hypocotyl may have started to respond first to the phototropic stimulus and that this was subsequently reversed by the antagonistic organotropic response directed by the cotyledon.

The postulated interaction between the organotropic and phototropic responses is supported by results showing that when the hypocotyl was in darkness, its curvature was negatively organotropic and of equal magnitude, whether the lamina of the remaining cotyledon was pointing towards the source of unilateral illumination (Fig. 5F) or away from it (Fig. 5E).

The direction of the light beam intercepted by the cotyledonary lamina can also modify the organotropic response of the hypocotyl. The darkened hypocotyl of an explant exhibited negative organotropism by curving away from the single cotyledon when its lamina was in darkness (Fig. 7C) or illuminated by a vertical beam (Fig. 7B) or by an oblique beam directed towards its tip (Fig. 7D). When the oblique beam was directed towards the base of the lamina, the organotropism was reversed and the hypocotyl curved toward the cotyledon (Fig. 7E). Figure 9, which compares the kinetics of these effects, also indicates that, whereas a base-oriented beam reversed the organotropic effect, a tip-oriented beam and a vertical beam enhanced it (to the same extent), as compared to darkness. Excitation of a blue-absorbing, vectorial photoreceptor in the cotyledonary lamina affects the negative organotropic curvature of the hypocotyl (Fig. 12). The same photoreceptor may be involved in the reorientation of the lamina normal to the light beam (9). However, curvature of the hypocotyl is a result of differential extension growth (Fig. 3), whereas laminar reorientation is a result of differential turgor in the pulvinus (13).

Extension growth is by nature auxin-dependent. We have previously suggested that: (a) in the mature leaf a darkened segment of the lamina induces "dark turgor" in the associated sector of the pulvinus, by means of polar transport of auxin to it; (b) exposure of such a leaf segment to a base-oriented beam leads to loss of turgor in the associated pulvinar sector, whereas its exposure to a tip-oriented beam leads to increased turgor; and (c) that these
turgor changes may be mediated by modification of polar transport of auxin through the veins, a reduction in the first case and enhancement in the second (9). Assuming that auxin is transported from the cotyledon and eventually reaches the subtending sector of the hypocotyl, it may also be responsible for the negative organotropic curvature when the lamina is in darkness. Inhibition of polar transport by base-oriented excitation of the lamina is responsible for the reversal of the organotropic curvature and its enhancement by tip-oriented excitation increases this curvature. In nongrowing pulvinar tissue, auxin is involved in the control of cell water potential, probably by regulating the metabolic uptake of cations. It is therefore likely that the level of auxin reaching the hypocotyl controls its elongation by determining not only cell wall extensibility, but also the osmotic component of the driving force for cell expansion.

Exposure of the cotyledonary lamina to a vertical beam enhanced the organotropic curvature of the hypocotyl to the same extent as its exposure to a tip-oriented beam (Fig. 9) and increased pulvinar turgor appreciably as well (Fig. 7). In darkness the pulvinar turgor is such that the lamina is partially declined. We therefore assume that laminas of vertically illuminated cotyledons remain horizontal because their vectorial photoreceptors are actually excited in this condition.

It is only when the lamina is fixed so that it is continuously excited by the base-oriented beam that growth of the subtending sector of the hypocotyl is inhibited to the extent that its negative organotropic curvature is reserved. This does not take place when the lamina is free to reorient normal to the beam (Fig. 8). This explains why nonilluminated hypocotyls of explants with two cotyledons showed positive curvature only when both laminas were continuously excited by the same oblique beam, but not when they were free to reorient normal to it (Fig. 11).

Results in Figure 10 show the integrated effects of phototropic excitation of the hypocotyl with those of vectorial excitation of the lamina. It appears that when the hypocotyl is unilaterally illuminated on the side from which a cotyledon has been removed, its phototropic response is maximal and therefore independent of vectorial excitation of the remaining cotyledon or of its being in darkness. If the hypocotyl is unilaterally illuminated on the side with the remaining cotyledon, the response is the same when the lamina is in darkness or exposed to a tip-oriented beam but is increased when the lamina is vectorially excited by a base-oriented beam. In other words, base-oriented vectorial excitation reduces the negative organotropic resistance to the phototropic response. The experiments summarized in Figure 11 show that in the absence of unilateral organotropic stimulation, curvature of the unilaterally illuminated hypocotyl of an explant with two cotyledons was dominated by its own phototropic response. Continuous excitation of the vectorial photoreceptor in both cotyledonary laminas by the same oblique beam resulted in a slight enhancement of the phototropic curvature of the hypocotyl exposed to the same beam. However, when the hypocotyl itself was in darkness, it exhibited considerable positive curvature in response to such vectorial excitation.

LITERATURE CITED