

Communication

Influence of Hook Position on Phototropic and Gravitropic Curvature by Etiolated Hypocotyls of *Arabidopsis thaliana*¹

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ABSTRACT

Phototropic and gravitropic curvature by hypocotyls of *Arabidopsis thaliana* is minimal when the side of the hook with the cotyledons attached is positioned toward the direction of tropistic curvature, and maximal when that side of the hook is positioned away from the direction of tropistic curvature. Based on these data, it is proposed that the position of the hook with attached cotyledons affects curvature and not stimulus perception. A randomly oriented population of plants exhibited considerable heterogeneity in tropistic curvature. This heterogeneity arises at least in part from the dependence of curvature on the position of the hook.

In studies of phototropism and gravitropism in plants, one typically measures the angle of curvature resulting from a particular stimulus. We have previously noted that frequency distributions of the curvature of a population of plants may contain information which is not evident from values of the mean and standard error alone (2). For example, there are usually some individuals in a frequency distribution showing zero curvature although the mean curvature of the population may be 90°.

We have recently observed that the angle of curvature, developed by the hypocotyl in response to a phototropic or gravitropic stimulus, depends on the position of the cotyledons or hook around the seedling axis with respect to the direction of curvature. Based on that observation, this study was undertaken to measure phototropism and gravitropism as a function of hook or cotyledon position.

MATERIALS AND METHODS

Plant Growth and Stimulation

Seeds of *Arabidopsis thaliana* (L.) Heynh., cv Estland, an epigeal dicot, were sown and the seedlings grown as previously described (8), with slight modifications. Strips of microassay

wells containing 0.8% agar supplemented with 1.0 mM KNO₃, were sown with one seed per well. The strips were placed in transparent plastic boxes which were sealed with parafilm, and kept for 3 d in darkness at 4 ± 1°C to potentiate germination. The strips were then exposed to continuous white light (20 h for the phototropism experiments; 7 h for gravitropism experiments) at 24 ± 1°C (7), and then moved back into darkness for 42 h at 25 ± 0.5°C until stimulation.

For phototropic stimulation, the rows of seedlings were removed from the plastic boxes, and exposed to five flashes of 450-nm unilateral light at 0.38 μmol m⁻² s⁻¹ for 0.26 s at 15-min intervals. Following the light treatment, the seedlings were returned to the incubation boxes and kept in darkness for an additional 1 h at 24 ± 1°C and 90% RH. All manipulations were made in complete darkness since green light has previously been shown not to be phototropically 'safe' (9).

For gravitropic stimulation, the seedlings were grown in the same manner as for phototropism, but after the 42 h in darkness, the seedlings were exposed to red light at 2.4 μmol m⁻² s⁻¹ for 1 h. The rows of seedlings were then turned onto their sides for 3 h of gravistimulation.

The light and gravitational stimuli were suboptimal in order that curvature be maintained at less than 90°, permitting the hook position to be easily measured from above, and to ensure that the response was not saturated.

Light Sources

White light used for potentiation of germination was provided from white fluorescent tubes at 125 μmol m⁻² s⁻¹ (General Electric DeLux). Light for phototropic stimulation was obtained from a slide projector equipped with a 300-W ELH multimirror quartzline bulb in combination with a 12-cm thick 1.25% (w/v) aqueous cupric sulfate solution. Wavelength was defined with an interference filter with a 10-nm half-bandwidth (PTR Optics, Waltham, MA) and peak transmission at 450 nm. The fluence rate was measured with an IL 700 A research radiometer (International Light, Newburyport, MA). The duration of actinic irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY). Red light was from a Sylvania Gold F15T12-GO tube (GTE Products Corp., Danvers, MA) filtered through a red cellophane transmitting light in the 560 to 720 nm range with peak transmission at 630 nm.

¹Supported by the U.S. Department of Energy under contract DE-AC02-76ERO-1338, and by the U.S. National Aeronautics and Space Administration under grant NAGW-882.

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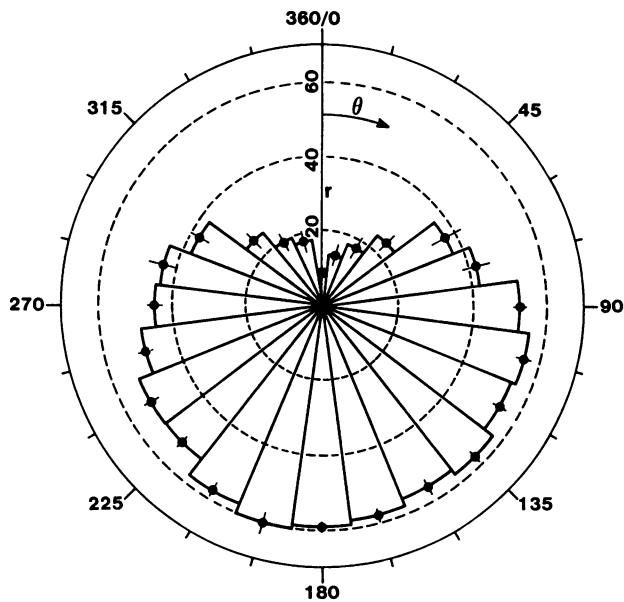


Figure 1. Effect of cotyledon position on phototropic curvature. The angle, θ , ranging from 0 to 360°, represents cotyledon position with respect to the direction of subsequent curvature. $\theta = 360/0$ represents plants with the cotyledon-attachment side of the hook toward the blue light; $\theta = 180$ represents plants with the cotyledon-attachment side of the hook positioned away from the blue light. The radius, r , ranging from 0 to 60°, represents the angle of curvature of the hypocotyl in response to the blue-light stimulus. Each 15° pie-shaped segment represents the mean phototropic curvature of the hypocotyls of all seedlings with cotyledons positioned within the range of that segment. All segments have $n > 10$. The bars represent ± 1 SE. Total number of plants at all cotyledon positions = 1514.

Measurement of Hook Position and Curvature

At the end of each experiment, the rows of seedlings were positioned under a Gaertner Scientific Corporation (Chicago, IL) goniometer. The position, with respect to the stimulus direction, of the cotyledons of each seedling around the plant axis was recorded. Since the cotyledons are easily seen and are attached at the end of the hook and point away from the main axis of the plant, cotyledon position is a convenient measure of the orientation of the hook around the plant axis. The recorded positions of the cotyledons varied from 0 to 360°. The seedlings were then gently adhered to a sticky transparent tape, keeping the direction of bending in the plane of the tape surface. The tape was then inserted into a photographic enlarger and the images of seedlings traced. For each seedling, the angle of hypocotyl curvature was measured from these enlarged images, and recorded along with the cotyledon position around the plant axis of that seedling.

Frequency Distribution Histograms and Polar Coordinate Graphs

Frequency distribution histograms showing the various populations of plants have been constructed and printed using VP-planner (Stephenson Software Inc.). To show the relationship between tropistic curvature and cotyledon position, polar coordinate histograms were constructed with tropistic curva-

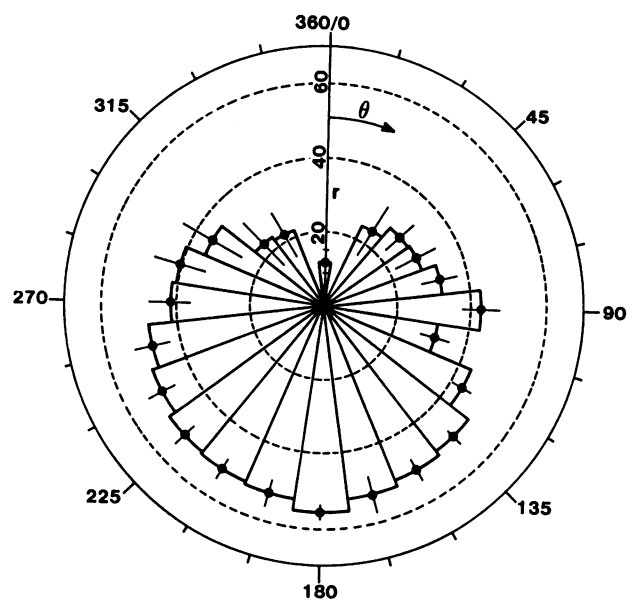


Figure 2. Effect of cotyledon position on gravitropic curvature. The angle, θ , ranging from 0 to 360°, represents cotyledon position with respect to the direction of subsequent curvature. $\theta = 360/0$ represents plants with the cotyledon-attachment side of the hook facing up during gravistimulation; $\theta = 180$ represents plants with the cotyledon-attachment side of the hook facing down during gravistimulation. The radius, r , ranging from 0 to 60°, represents the angle of curvature of the hypocotyl in response to the gravitational stimulus. Each 15° pie-shaped segment represents the mean gravitropic curvature of the hypocotyls of all seedlings with cotyledons positioned within the range of that segment. All segments have $n > 10$. The bars represent ± 1 SE. Total number of plants at all cotyledon positions = 757.

ture plotted as a function of cotyledon position around the plant axis.

RESULTS AND DISCUSSION

It is evident that phototropic or gravitropic curvature of the hypocotyls is affected by cotyledon position around the plant axis (Figs. 1 and 2). Phototropic curvature of a seedling is greatest when the side of the hook with the cotyledons attached is positioned away from the incident light direction, or away from the direction of subsequent curvature (Fig. 1). Gravitropic curvature is greatest when the side of the hook with the cotyledons attached is positioned toward the gravitational stimulus, or away from the direction of subsequent curvature (Fig. 2).

If one had only the data for phototropic curvature as a function of cotyledon position, it would be tempting to suggest an optical basis, such as screening, for the phenomenon. Such an optical basis has been suggested to account for the dependence of phototropic curvature on orientation of coleoptiles. Meyer (3) reported a slight increase in the curvature of *Avena* when an actinic beam of blue light passed along the long transverse axis through the vascular bundles. However, the data presented here for *Arabidopsis thaliana* also show a dependence of gravitropism on cotyledon position, and an optical mechanism could not be its basis. Unless two totally

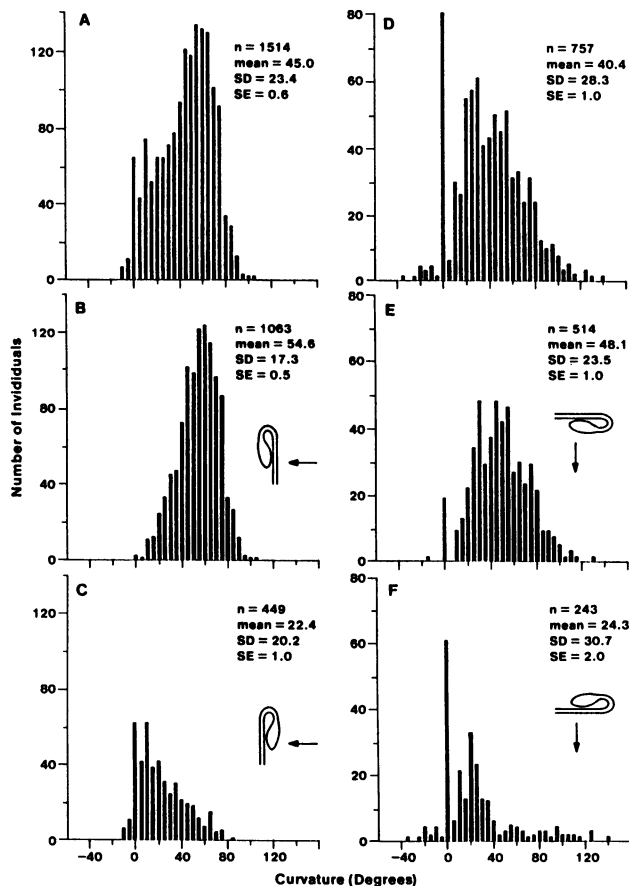


Figure 3. Frequency distributions for tropic curvature. A, B, C, phototropism; D, E, F, gravitropism. A, D, total population, irrespective of cotyledon position. B, E, the portion of the total population of plants with their cotyledon-attachment side of the hook positioned at $\theta = 180 \pm 90^\circ$ (away from the direction of subsequent curvature). C, F, the portion of the total population of plants with their cotyledon-attachment side of the hook positioned at $\theta = 360/0 \pm 90^\circ$ (toward the direction of subsequent curvature). The small seedling diagrams on B, C, E, and F show the position of the cotyledons relative to the stimulus vector (arrow) for individuals in that distribution.

separate mechanisms give rise to the similar dependences of phototropism and gravitropism on cotyledon position, an optical mechanism for this effect in *A. thaliana* must be rejected.

The measurement of light direction by plants requires a spatial gradient within the plant in light intensity (5, 6). Since the 1g force of gravity is pervasive on earth, the measurement of the direction of the gravitational force cannot involve a spatial gradient of gravitational force. Thus, if a single mechanism accounts for the dependence of both phototropism and gravitropism on cotyledon position, then that mechanism cannot include a spatial gradient of the stimulus. Moreover, it should be noted that the hypocotyl exhibits positive phototropism but negative gravitropism, so maximum curvature is not a function of hook position with respect to the stimulus direction. Thus, we believe that the effect of hook position is not on stimulus perception. Rather, maximum curvature is a function of cotyledon position with respect to subsequent curvature.

It seems likely that the effect of cotyledon position on subsequent curvature is *via* the transmission or modulation of some signal along the hypocotyl with different rates or extents on the sides toward and away from the subsequent curvature. Any conjecture concerning the nature of that stimulus would be completely speculative at this time. Although one's first thought might be of a biochemical (*e.g.* hormonal) stimulus, the idea of a biophysical (*e.g.* electrical) signal should not be ignored. For example, the transmission of a biophysical stimulus from the hook has been proposed for mustard (4). This effect of cotyledon position on subsequent curvature could be related to ethylene-induced growth, which also depends on hook position (1).

One positive and practical consequence of this observation is the identification of at least one component of the heterogeneity found in the response of any population of *A. thaliana* plants to blue light or to gravity. If all of the individuals are pooled for one frequency distribution histogram for phototropism (Fig. 3A), and another histogram for gravitropism (Fig. 3D), each distribution shows considerable heterogeneity. However, the results are quite different if the same individuals are divided into two subpopulations dependent upon the position of the cotyledons relative to the main axis of the seedling. The frequency distribution histograms for the plants with the side of the hook with the cotyledons attached pointing toward, $\pm 90^\circ$, the direction of subsequent curvature (Fig. 3C, F for phototropism and gravitropism, respectively) show populations with very little curvature. In contrast, the frequency distribution histograms for the plants with the side of the hook with the cotyledons attached pointing away from, $\pm 90^\circ$, the direction of subsequent curvature (Fig. 3, B and E for phototropism and gravitropism, respectively) show populations with much greater curvature. Thus, we conclude that the frequency distribution histogram for the total population represents a composite of at least two subdistributions: one subdistribution with the cotyledon-attachment side of the hook away from the direction of curvature, and strong subsequent curvature; and a second subdistribution with the cotyledon-attachment side of the hook toward the direction of curvature, and weak subsequent curvature.

It will be interesting to study the effect of cotyledon position on phototropic and gravitropic curvature in other epigeal dicots. Certainly, the use of plant material larger than *A. thaliana* would facilitate the study of the nature of the stimulus transmitted along the hypocotyl from the hook. In any plants exhibiting this phenomenon, knowledge of the effect will permit one to eliminate at least one source of heterogeneity in the phototropic or gravitropic response.

ACKNOWLEDGMENTS

We are indebted to B. Bullen, M.-C. Fortin, R. Konjević, V. Orbović, and R. Perry for their helpful comments.

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