Stem Sensitivity and Ethylene Involvement in Phototropism of Mung Bean

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ABSTRACT

A system is described for the examination of phototropism in the epicotyl of a dicot seedling, mung bean (Phaseolus aureus Roxb.), under conditions approximating nature, including the use of intact, nonetiolated plants exposed to elevated, continuous, white, unilateral light. It is found that in this system perception of the phototropic stimulus by the leaves alone cannot account for the curvature, and that exposure of the stem is also necessary. The phototropic response was found to be strongly altered in nonintact plants. Hypobaric treatment indicates that ethylene may participate in phototropism, possibly by acting as an inhibitor of auxin transport.

Dose-response studies show that different types of phototropic curvature are expressed at different levels of light: (a) the first positive, induced by exposure for short periods of time; (b) a negative or indifferent response, induced by higher doses of light; and (c) the second positive, which is brought about by exposures of longer duration. This general pattern seems to apply to both monocots (5) and dicots (18). The similarities and differences between the mechanisms which mediate these different responses have not been clearly elucidated, and it is possible that several completely different mechanisms are involved. Curry (5) proposed a model which could account for first positive, first negative, and second positive curvatures under a single scheme.

The type of curvature induced by exposure to longer periods of light is the one which is important under natural conditions, and the one which we chose to study. There is some evidence to indicate that plants grown under natural environmental conditions may not respond in exactly the same way as those subjected to certain laboratory treatments. For example, Everett (6) found that the dose-response curve for radish seedling phototropism is different for dark-grown and light-grown plants. Shaw et al. (15) suggested that the patterns of auxin movement observed in explants subjected to tropistic stimuli may be different than the movement in intact plants. This points to a need for the study of phototropism under relatively natural conditions. In nature, phototropism takes place in intact plants which are grown under alternating periods of light and darkness and exposed to elevated, white, unilateral light for periods of several hours at a time. We have attempted to simulate these conditions in our experiments.

Previous research on dicot phototropism (11, 17) led to the conclusion that the phototropic stimulus is perceived primarily by the leaves and cotyledons. Our experiments indicate that leaf sensitivity is not of universal occurrence among dicots. Our results demonstrate that in mung bean seedlings the primary zone of perception is the stem.

It is generally agreed that phototropism is caused by the accumulation of auxin on the dark side of the curving organ. Gordon and Shen-Miller (9) have suggested that auxin asymmetry in coleoptiles is caused by impairment of basipetal auxin transport on the light side. We have performed experiments under hypobaric conditions which indicate that light-induced ethylene might participate in causing such transport inhibition in mung bean.

The three primary objectives of these experiments were (a) to develop a system approximating natural conditions for studying phototropism in a dicot; (b) to determine the photosensitive zone of the mung bean seedling; and (c) to examine the involvement of ethylene as a regulatory factor in phototropism.

MATERIALS AND METHODS

Plant Material. Seeds of Phaseolus aureus Roxb. were surface-sterilized by soaking them for 5 min in 5% Clorox solution. The seeds were then rinsed and soaked in continuously aerated tap water for 24 hr. The aeration served both to provide O₂ and to help dislodge the seed coats. The water was changed after approximately 6 and 18 hr. At the end of the 24-hr period the germinating seeds were removed, rinsed, and planted 0.5 cm deep in medium-grain perlite in 5 cm square plastic pots, four seeds per pot. The plants were grown in a growth chamber under 16 hr light and 8 hr dark, 28 C/20 C, and 65% relative humidity. The light intensity in the chamber was 1500 ft-c provided by incandescent and fluorescent lamps. Plants 7 days old (from the time when inhibition began) and 5 cm ± 3 mm in height with straight epicotyls were used for experimental purposes. Only one plant per pot was selected; the other three were discarded. At this stage, the primary leaves were fully developed, but the secondary leaves were still embryonic.

Phototropic Illumination. A Precision Scientific Co. oven (inside dimensions 30 × 20 × 20 cm) lined with black paper was used as an illumination chamber. The chamber had a glass door and gas inlet and outlet valves. The light source was a PBL Lab-source QH 150 quartz halogen illuminator equipped with an HM-A90-012-M heat reflecting filter and an HAF-01-M heat absorbing filter. The intensity dial on the instrument was set on “L,” giving an intensity of 650 ft-c in the chamber. The lighting configuration is shown in Figure 1. Unilateral illumination was applied continuously for the periods of time indicated. The plants were arranged in the chamber in two rows of three each, still in the pots, with the pair of primary leaves oriented perpendicular to the direction of the light except where otherwise indicated. During the experiments, fresh air was continuously...
circulated through the chamber, and the temperature was maintained at 25°C.

**Measurement of Curvature.** At the end of the exposure period, the plants were removed from the chamber, cut off just above the soil line, and the leaves and cotyledons were removed. The curvatures were confined to the epicotyl region of the stem. The stems were immediately placed on a glass plate and projected onto Kodak F3 projection paper to produce a shadowgraph enlarged to approximately twice actual size. Tracing paper was then placed over the enlarged images, tangent lines were drawn to the two portions of the stem, and the angle of curvature was measured with a protractor. Attempts to measure the curvature directly from the plant or from an unenlarged image were unsatisfactory.

Since the chamber held 6 plants at a time, each experimental treatment was run twice, for a total of 12 plants. The highest and lowest curvatures were dropped, leaving 10 values. The curvatures presented herein are the averages of those 10 values.

**Masking Experiments.** Masking of the primary leaves and the stem was done with aluminum foil. When the stem was masked, it was covered from the soil line to a height of 1 to 2 mm below the point of attachment of the primary leaves. The foil was arranged loosely to prevent mechanical interference with curvature. Because of its confined location between the primary leaves, the step tip (apical meristem plus embryonic leaves) was masked with a black mixture of lanolin and animal charcoal, both of which are inert to the plant.

**Hypobaric Treatment.** For the experiments involving hypobaric conditions, the chamber was maintained at one-tenth atm and was ventilated with a gas mixture containing 0.3% CO₂ and the balance O₂. The partial pressures in the chamber then corresponded to 0.03% CO₂ and 9.97% O₂ at atmospheric pressure. Gas levels were measured by gas chromatography. An open Petri dish of distilled H₂O was kept in the chamber to maintain the humidity, and the plants remained fully turgid at all times.

**RESULTS**

**Photosensitive Zone.** To determine the zone of the plant which perceives the phototropic stimulus, different parts were

masked, the plants exposed to unilateral light for 2 hr, and the resulting curvatures measured. The results are shown in Table 1.

Treatment 2 was oriented with leaves parallel to the direction of the light rays (Fig. 1B). All others were in the perpendicular orientation. In treatment 2, unmasked plants with leaves parallel had an average curvature 5 degrees less than the unmasked perpendicular control, and the response was more variable. This was probably due to some shading of the stem and tip by the leaves in the parallel arrangement. Therefore, we decided that the perpendicular orientation is preferable for experimental use.

Masking the primary leaves caused only a 25% reduction in curvature, indicating that they were probably not playing a leading role in sensing the phototropic stimulus. Masking the tip reduced curvature by more than half, but when the stem itself was masked no curvature developed. The stem-masking experiment was repeated with an exposure time of 5 hr, and still there was no curvature.

In experiments with decapitated sunflower seedlings (11), the investigators achieved curvature by masking or removing one cotyledon and exposing the plants to bilateral light in a growth chamber for 5 hr. A similar experiment was performed with intact mung bean seedlings with one primary leaf masked, but no measurable curvature developed. This further suggests the dissimilarity between the methods of perception in sunflower and mung bean, the latter apparently not being mediated by the leaves.

Attempts were also made to determine the photosensitive zone by the method of removing various plant parts. It was found that the removal of either one or both primary leaves or the stem tip caused almost complete inhibition of phototropic curvature.

**Effect of Hypobaric Treatment.** Plants were exposed in the perpendicular orientation to continuous unilateral light for periods of 1, 2, 3, and 4 hr under both atmospheric pressure and one-tenth of an atmosphere, and the resulting curvatures were measured (Fig. 2). Decreased curvature was observed under hypobaric conditions at all illumination times when compared to curvatures at one atmosphere. Statistical analysis of the data showed these differences to be significant at the 99% confidence level for the 2 and 3 hr periods, and at the 90% level for 1 and 4 hr.

**DISCUSSION**

Previous work (11, 17) with dicot (sunflower) seedlings led to the conclusion that the leaves or cotyledons are the photosensitive parts. Other authors concluded that the leaf nearest the light source receives the most intense light and that this causes inhibition of auxin transport out of that leaf, resulting in a higher concentration of auxin on the dark side of the stem (11). However, in nature, such a gradient of light intensity across the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Curvature</th>
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<tbody>
<tr>
<td>1. Unmasked, leaves perpendicular</td>
<td>25.2 ± 5</td>
</tr>
<tr>
<td>2. Unmasked, leaves parallel</td>
<td>20.2 ± 6</td>
</tr>
<tr>
<td>3. Primary leaves masked</td>
<td>18.7 ± 3</td>
</tr>
<tr>
<td>4. Tip masked</td>
<td>11.2 ± 3</td>
</tr>
<tr>
<td>5. Stem masked</td>
<td>0</td>
</tr>
<tr>
<td>6. Tip or leaves removed</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Effect upon Phototropic Curvature of Masking or Removing Plant Parts

Plants were exposed to 2 hr of unilateral light. Leaves were parallel to light rays in treatment 2, all others were perpendicular.
leaves probably does not exist. In fact, the leaf on the side of the seedlings away from the sun may actually be receiving the most intense light. This may be seen by considering the morphology of a mung bean seedling and the geometry of the sun’s illumination in relation to it (Fig. 3). The sun may be considered a point source of light at an infinite distance, and its light rays assumed parallel. If \( \theta \) is the angle at which the light rays strike the leaf at a given time of day, the vertical component of the illumination vector is proportional to \( \sin \theta \). The leaves of the young seedlings are often at an acute angle to the stem as shown in Fig. 3A. In this orientation \( \theta_1 \) is greater than \( \theta_2 \) and the leaf on the side away from the sun receives the most illumination. If the leaves were at right angles to the stem, then \( \theta_1 \) would equal \( \theta_2 \) and there would be no light gradient at all. Also, in the perpendicular orientation (Fig. 3B) \( \theta_1 \) equals \( \theta_2 \) and no gradient exists. In the case of sunflower cotyledons, the tips sometimes droop down and the one nearest the sun could conceivably receive more light in the parallel orientation, but this would still not account for the situation in the perpendicular arrangement. It does not seem that a gradient of light across the leaves or cotyledons (sunflowers have leafy cotyledons, mung beans do not) can account for sensing a phototropic stimulus. The place where a light gradient does exist is on the stem and its tip, and our results indicate that these are the primary zones of perception in mung bean seedlings.

Part of the basis for the assumption that the leaves of sunflowers are the sensitive parts was the observation that removal of the leaves inhibited curvature (17). Whereas the removal of leaves or tip also caused almost complete inhibition of curvature in mung bean, the masking of these same parts did not cause that much inhibition. The excision of these organs is apparently causing some sort of alteration in the phototropic response of the mung bean seedling. This should be examined in more detail in order to determine the nature and extent of the interference, and to assess the validity of experiments performed with nonintact plants.

The tip shows some degree of sensitivity when it alone is masked (Table I). This sensitivity seems to be lost when the stem is no longer exposed. It could be that an internal gradient of growth promoter (presumably auxin, although this has not yet been verified) becomes partially established in the tip. The stem, however, is where curvature is ultimately expressed, and when it is masked the gradient within the stem cannot be maintained and no curvature occurs.

Several theories have been proposed to explain the formation of the asymmetric auxin distribution found in phototropism. The two most prominently mentioned in the current literature are lateral transport and inhibition of basipetal transport. Gordon and Shen-Miller (9) and Gardner et al. (8) have shown that light-induced inhibition of basipetal auxin transport may be functioning in phototropism of monocots, including the second positive response. It is possible that a similar system exists in mung bean. Lateral transport takes place in peas, but van Overbeek (19) and Lam and Leopold (11) have shown that it does not occur in all dicots. Light does inhibit the basipetal movement of auxin in both monocots (14) and dicots (16). Naqvi (13) suggested that the inhibition in Zea coleoptiles may be due to light-stimulated abscissic acid synthesis. Ethylene also inhibits auxin transport (12). This effect is sometimes not observed until after several hours of exposure to applied ethylene, but the growth-inhibiting effect of ethylene on pea seedlings, which may also be due to transport inhibition, is seen in as little as 6 min (20). Response to endogenous ethylene may, of course, be even more rapid than to the applied gas. The action spectrum for ethylene biosynthesis determined by Craker et al. (4) resembles the action spectrum for phototropism in that both are induced primarily by blue light. Ethylene biosynthesis also exhibits a narrow peak in the far red, which phototropism does not. These action spectra were determined for other organisms, so we are only speculating that they might be similar in mung bean. It was observed that light of 450 nm wavelength was effective in inducing curvature (data not shown), indicating that blue light is phototropically active in mung bean, as it is in other plants. Also, the production of ethylene from methionine in the presence of flavin mononucleotide is stimulated by light (21). This could be significant, in view of the fact that the flavins have been implicated as possible receptor pigments in phototropism (7).
Most auxin is synthesized in the meristematic cells of the shoot apex and is transported down into the stem. If ethylene were to be synthesized at a faster rate on the light side of the stem it could diminish the flow of auxin into this region and result in a higher concentration of auxin on the dark side. This would be consistent with our finding that exposure of the stem is necessary in order for curvature to develop. In addition to inhibiting auxin transport, ethylene also inhibits cell elongation (2), which could account for the decreased growth rate found on the light side of a stem undergoing phototropic curvature (11).

In contrast, higher ethylene levels have been found on the dark side of a phototroping stem (1), but this was after a 5-hr time interval and was probably the result of an auxin gradient which had already existed for some time.

Hypobaric treatment is used to remove endogenous ethylene rapidly (3), thereby causing a decrease in ethylene-mediated responses. The depression of curvature under hypobaric conditions (Fig. 2) indicates that phototropism in the mung bean seedling could depend at least in part on ethylene. These results should be interpreted with caution because it is uncertain what effects hypobaric treatment might have in addition to ethylene removal. Kang and Burg (10) reported that hypobaric treatment had no effect on phototropism in pea stems. However, these experiments were performed on excised sections of etiolated plants at a different pressure and with a longer exposure time, so their results and ours are not comparable. Additional work is required to test the hypothesis that ethylene may be involved in phototropism.

We conclude that perception of the phototropic stimulus in mung bean seedlings is primarily by the stem. Phototropic curvature is inhibited under hypobaric conditions, possibly indicating the involvement of ethylene in mediating the response. The removal of plant parts alters the normal phototropic response in this system.