Short Communication

Effect of Red Light on Geotropism in Pea Epicotyls

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ABSTRACT

Dose response curves were determined for phytochrome phototransformation and for a phytochrome-controlled decrease in geotropic curvature in epicotyls of dark-grown Pisum sativum L. cv. Alaska. Ten times as much light was required to produce a spectrophotometrically detectable transformation of phytochrome as was required to produce a significant change in the geotropic response. The red light energy required for a 50% phytochrome transformation caused a 90% change in the physiological response.

There is evidence that not all spectrophotometrically detectable phytochrome must be transformed to the Pfr form for saturation of various physiological responses (2, 5). The light energy necessary to saturate a shift in physiological sensitivity of corn phototropism was more than two orders of magnitude less than the amount (about $1 \times 10^{-3} \text{E} \text{cm}^{-2}$) required to transform a detectable amount of phytochrome (1). The irradiance of R² needed for a threshold effect on corn phototropism ($1 \times 10^{-12} \text{E cm}^{-2}$) actually exceeded by a factor of 3 the irradiance needed for a threshold effect on the geotropic response of oat coleotilpes ($3.3 \times 10^{-12} \text{E cm}^{-2}$) (12). Several studies with Alaska pea seedlings have indicated that there are discrepancies between the amount of detectable phytochrome phototransformation or the measurable amount of Pfr and the degree of physiological response (4, 5).

The purpose of the present study is to determine whether phytochrome can in some way affect geotropic sensitivity in pea epicotyls and if so, what levels of Pfr are required. A related question is whether the large discrepancies mentioned above for the amounts of Pfr required for physiological action in corn and oat tropisms versus the total phytochrome present were a peculiarity of coleotilpes, or could be detected in other tropic systems as well.

MATERIALS AND METHODS

Alaska peas (Pisum sativum L. cv. Alaska, obtained from Burpee Seed Co., Riverside, Calif.) were germinated and grown in darkness for 4 days in glass vials containing 1% agar. A group of seedlings of similar size (epicotyl height 2.4–3.2 or 3.3–3.8 cm) was then selected. At appropriate times after R treatments, racks containing 10 plants were placed with the epicotyl hooks horizon-
tal for geotropic stimulation (usually for 160 min). At the end of the stimulation period the seedlings were shadowgraphed with Kodabromide F-4 photographic paper (Eastman Kodak, Rochester, N.Y.). Before shadowgraphing all seedlings were rotated 90° so that the geotropic curvatures developed would all be in the same plane.

For spectrophotometric determination of the relative amount of transformed phytochrome, epicotyls selected as for geotropic stimulation were placed in a cold room (4°C) for a 2-hr equilibration period. They were then irradiated in the identical fashion as for the geotropic experiments. Immediately after irradiation, samples of five whole epicotyls were harvested, chopped into pieces 1 to 2 mm in length, and placed in a chilled cuvette in a dual wavelength spectrophotometer (Ratiomespect R-2, Agricultural Specialties Co., Md.) to determine the amount of phytochrome transformation. Less than 5 min were required to harvest, prepare, and measure the samples. Following harvesting, they were kept on ice and exposed only to dim green light to eliminate any significant dark or light reactions of phytochrome during sample preparation.

For preliminary determination of the effect of R on geotropism, and for determination of the time course of the R response, as well as for examination of FR reversal, broad spectrum R and FR sources were used. The R source consisted of light from cool-white fluorescent tubes filtered through red Plexiglas (Rohm & Haas 2423, 3 mm thick, Corh Plastics, Redwood City, Calif.). This source provided an intensity of 870 ergs cm⁻² sec⁻¹ at plant level. The FR source consisted of four 60-W incandescent bulbs plus far red Plexiglas (Rohm & Haas FRF, 3 mm thick, Corh Plastics) and a 3-cm water filter. The intensity at plant level was 2,600 ergs cm⁻² sec⁻¹. Intensities were measured with an Eppley Laboratory 8-junction bismuth-silver thermopile (No. 3975) in conjunction with a Hewlett-Packard microvoltmeter (model 425A). Corrections for IR light were made by measuring the intensity with and without a Polaroid Corp. filter (XRN-5X55) which transmits approximately 80% beyond 900 nm and less than 1% below 800 nm. The difference represents the amount of IR beyond 900 nm which needs to be subtracted from the reading obtained without the Polaroid filter to determine the energy of light within the biologically effective wavelength range, either R or FR.

The R source for determination of the dose response relationships both for the physiological response and for phytochrome phototransformation was a Bausch & Lomb 33-86-07 grating monochromator with a 100-W tungsten halogen lamp. The monochromator was set at 670 nm with the exit slit at 1.56 mm. Stray light was reduced and higher order wavelengths eliminated with a Corning 3-69 cut-off filter. The correction for IR light was obtained with a Corning 7-56 IR-transmitting, visible-absorbing filter instead of the Polaroid XRN-5X55 filter as above.

All manipulations were under dim green light provided by 15-W fluorescent bulbs wrapped in two layers of amber and one layer.

1 This investigation was supported in part by National Science Foundation Grants GB-2846 and GB-6683 to W. R. Briggs. C.I.W.-D.P.B. Publication No. 632.
2 Abbreviations: R: red light; FR: far red light.
of green cellulose acetate (Shades, Inc., San Francisco). This filter combination showed greater than 1% transmission only between 522 and 563 nm. Humidity was about 90% and growth temperature was 25 ± 0.5°C.

**RESULTS**

The rate of development of geotropic curvature for pea epicotyls was determined in order to select a treatment producing maximum curvature. It was reasoned that with such a treatment, any effect of R might also be maximized. The development of curvature during 160 min of geostimulation is shown in Figure 1. Three things are clear from these results: (a) curvature development is almost linear at least for 2.5 hr; (b) curvature is maximal under these conditions at 160 min; (c) 3-min R given immediately prior to the beginning of geostimulation was without effect.

In the next experiments, R-treated plants were kept vertical for a dark incubation period prior to geotropic stimulation to determine whether a R-induced change in geotropic sensitivity might develop, and if it did, to determine what the time course for this development might be (Fig. 2). There was a substantial reduction in the magnitude of the geotropic response as a consequence of R treatment, but the effect required between 30 and 45 min to develop.

This R effect on geotropism was completely reversed by FR. Groups of plants of two different epicotyl lengths (2.4–3.2 cm or 3.3–3.8 cm) were either used as dark controls, irradiated with 5-min R (dose: 2.6 × 10⁶ ergs cm⁻²) or 5-min R followed by 10-min FR (dose: 1.6 × 10⁸ erg cm⁻²) (Table I). The 10-min FR irradiation was administered during the first 10 min of the post-R treatment incubation period, in these experiments, 30 min total. In both experiments, the effect of R was completely reversed by FR.

To obtain a dose response curve for the effect of R on the geotropic response, plants were given R (5.4 × 10⁻⁶ E cm⁻² sec⁻¹) for various times, kept vertical for 45 min in darkness, and then placed horizontal for geotropic stimulation and subsequent shadowgraphing as described above. Figure 3 shows that the light-inducible alteration of the geotropic response was saturated by about 300 sec or less of R, and a 50% response could be obtained with less than 30 s. It also shows the dose response relationships for phytochrome phototransformation. Saturation of phototransformation required 700 sec and a 50% response required about 160 sec. A statistically significant physiological response could be detected with as little as 1-sec R, while more than 10 sec were required to produce detectable phytochrome transformation. The dose response curve for the physiological response was obtained on three separate occasions, as was that for phytochrome phototransformation.

The pea epicotyl hook contains a much higher concentration of phytochrome than any other part of the seedling (6). To improve the sensitivity of the measurement of phytochrome transformation, samples consisting only of hooks were harvested following R treatment. A R exposure of 10 sec still failed to produce any measurable phototransformation. These results represent further evidence that only a small fraction of the total phytochrome need be transformed to Pfr to yield saturation of the physiological response, a change in geotropic sensitivity. A R dose insufficient to transform measurable phytochrome is nevertheless enough to produce over 40% of the possible physiological response. Sufficient R to give 50% phytochrome transformation is almost saturating for the change in geosensitivity (Fig. 3).

**DISCUSSION**

There are numerous studies of the effects of R on the tropic responses of dark-grown seedlings. On the one hand, R increases the sensitivity of the second positive phototropic response of corn (1, 3) and oat (13) coleoptiles, increases the geotropic reactivity of etiolated mustard seedlings (7), and increases the geosensitivity both of *Convolvulus* (11) and corn (9) roots. R decreases the sensitivity of the first positive phototropic response of corn (3) and oat (13) coleoptiles, decreases the geotropic reactivity of *Tropaeolum* shoots (10), and decreases the geosensitivity of the first internode of etiolated pea seedlings (present work). Until now, only in corn have dose response curves been obtained in parallel for R-induced alteration of tropic sensitivity and for phytochrome phototransformation (1). As mentioned above, the results showed that only a minute fraction of the total phytochrome need be transformed to Pfr to saturate the response. The threshold R dose necessary to alter geotropic sensitivity in oats (12) is even lower than that for alteration of phototropic sensitivity in corn. Inasmuch as there is no reason to expect the extinction coefficients and quantum efficiencies for corn and oat phytochrome to differ significantly (8), the same discrepancy between light needed to bring about physiological change versus that needed to transform phytochrome undoubtedly obtains with oat geotropism. The threshold light dose reported by Tepfer and Bonnet (11) for potentiating a change in the geosensitivity of *Convolvulus* roots was less than 10⁻¹⁴ E cm⁻² and it seems highly unlikely that such a small dose would transform measurable phytochrome.

The present work establishes, first, that phytochrome in some way modulates geotropic sensitivity in etiolated pea epicotyls, and, second, that as with corn phototropism, there need be very little phytochrome transformed to Pfr to yield a measurable physiolog-
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TABLE I. Far Red Light Reversal of the Red Light Effect

<table>
<thead>
<tr>
<th>Plant Size (cm)</th>
<th>Treatment</th>
<th>Number in Sample</th>
<th>Curvature (degrees ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 - 3.2</td>
<td>Dark Control</td>
<td>38</td>
<td>57 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>5 min R</td>
<td>40</td>
<td>47 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>5 min R +</td>
<td>59</td>
<td>56 ± 1.5</td>
</tr>
<tr>
<td>3.2 - 3.8</td>
<td>Dark Control</td>
<td>49</td>
<td>52 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>5 min R</td>
<td>40</td>
<td>44 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>5 min R +</td>
<td>40</td>
<td>51 ± 1.6</td>
</tr>
</tbody>
</table>

![Graph](image)

FIG. 3. Dosage response curves. Upper curve shows effect of R on geotropic curvature of the 2.8- to 3.2-cm pea epicotyl. Maximal inhibition obtained was 40% of the normal curvature of 63° (dark control); thus, "% R effect" represents a percentage of the 40% value (taken as 100%). Lower curve represents the per cent transformation of phytochrome by R as determined directly from Ratiospect values for Pfr and Ptot. Each of the curves combines the similar results of three experiments; bracket is standard error of the mean. R intensity: 5.4 × 10^{-11} E cm^{-2} sec^{-1}.

CITED

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4. FOX LR, WS HILLMAN 1968 Response of tissue with different phytochrome contents to various initial phytochromatic states. Plant Physiol 43: 823-826
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Physical response. This relationship obtains although the corn phototropic system is far more sensitive than the geotropic system of peas (irradiance for 50% response in corn: 3 × 10^{-12} E cm^{-2} (1); for 50% response in pea, 9.5 × 10^{-10} E cm^{-2}). Other than extending parallel measurements of transformation and potentiation of response to a plant organ other than a coleoptile, and showing that the same discrepancy exists, these experiments still do not form an adequate basis on which to build a testable model. The "Zea paradox" as discussed by Hillman (5) over 10 years ago is now formally extended to Pisum, but remains a paradox.