Light-controlled Leaf Expansion in Peas Grown under Different Light Conditions

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WILLIAM M. ELLIOTT
Department of Biology, Hartwick College, Oneonta, New York 13820

ABSTRACT

Several photosystems control leaf expansion in Alaska peas (Pisum sativum). Phytochrome is known to control expansion in dark-grown peas. But plants exposed briefly to red light are insensitive to phytochrome, an insensitivity that is itself phytochrome-produced. Leaf expansion in these plants is promoted by 440 or 630 nm of light (probably mediated by protochlorophyll). Plants grown in white fluorescent light required simultaneous exposure to high intensity blue and yellow light for promotion of leaf expansion. Since these results parallel studies on light-controlled inhibition of stem elongation, shoot growth as a whole is coordinated by these photosystems. Such coordination might be a mechanism of plant competition for light.

At least four pigments may inhibit stem elongation in peas, depending upon the light conditions under which the plants have been grown. (a) If plants are grown in darkness, phytochrome is the inhibiting pigment (13). (b) Protochlorophyll appears to be the pigment responsible for inhibition in plants exposed to red light (6). (c) Inhibition of stem elongation in plants grown under white fluorescent light is apparently the result of two HERs1 that must be activated together (6). I report that leaf expansion in pea seedlings is also controlled by protochlorophyll and two HERs under the same conditions as is stem elongation, indicating that these photosystems coordinate shoot growth under conditions in which the phytochrome system does not operate.

MATERIALS AND METHODS

Plant Material. Seeds of Pisum sativum cv. Alaska were grown in vermiculite for 6 days at 25 to 26 C under three regimes: (a) complete darkness (referred to as D-grown state), (b) complete darkness for 5 days followed by 12 hr red light (20 \( \mu \)W/cm\(^2\)) and 12 hr darkness (R-insensitive state), and (c) white fluorescent light (W-grown state). Plants grown in darkness were kept in an Environ Room (Lab-Line Instruments, Melrose Park, Illinois) located in a darkroom; light-grown plants were kept in a Percival PGC-7 growth chamber. After exposure to one of these regimes, series of parallel lines 2 mm apart were marked with India ink on the third or fourth pairs of leaves (9). Maximum increase in length between two lines was measured with an ocular micrometer after a 24-hr experimental period (Fig. 1). Because leaves of completely dark-grown plants were too small for this method, total leaf length was measured. It was not possible to determine the plastochron index of the leaves since they were all generally under 10 mm in length (11).

Light Sources. The blue, green, yellow, orange, and red light sources were obtained by filtering white fluorescent light through various combinations of cellulose acetate filters from Edmund Scientific Company (Table I in ref. 6). The distance between the level of the pea seedlings and the light source was varied so that the seedlings received a total dose of \( 5 \times 10^8 \) photons/cm\(^2\) over a 24-hr period from each of the five colors of light.

Light of greater purity was obtained by filtering the light from a 500-w tungsten halogen lamp (Edmund Scientific Company) through Bausch and Lomb interference filters combined with cellulose acetate filters to block unwanted transmission orders.

Each experiment, representing 10 separate measurements, was repeated three or four times. Values used are the means and standard errors of these experiments.

RESULTS AND DISCUSSION

Dark-grown State. Preliminary experiments indicated that leaf expansion in dark-grown pea seedlings was controlled by the pigment phytochrome, confirming earlier studies (13, 15). Dim R promoted leaf expansion; the promotion was reversed by FR.

Phytochrome-promoted leaf expansion in dark-grown peas is short lived. When dark-grown plants are exposed to continuous dim R (20 \( \mu \)W/cm\(^2\)), leaf expansion is promoted for about 24 hr then becomes insensitive to R, indicating loss of phytochrome control (Fig. 2). In W (3,000 \( \mu \)W/cm\(^2\)) growth is rapid and constant throughout a 40-hr period. There is virtually no leaf expansion in darkness.

The loss in sensitivity to R after 24 hr appears to be mediated by phytochrome. Plants exposed to 30 min of R and 24 hr of darkness are insensitive to a 24-hr exposure to dim R (\( \Delta L = 0.99 \) mm). If the 30 min of R is followed by 30 min of FR, full sensitivity to the 24-hr R treatment is restored (\( \Delta L = 2.42 \) mm compared with 2.50 mm for D control and 2.49 for FR control). Because the effect of the R pretreatment is reversed by FR, phytochrome control is indicated.

The phytochrome-mediated loss in R sensitivity might occur in two ways. An R exposure results in loss of detectable phytochrome (3, 5), which in turn could lead to loss of phytochrome.

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2 Abbreviations: HER: high energy reaction; B, G, Y, O, R, and FR: blue, green, yellow, orange, red, and far red light; W: white fluorescent light; \( \Delta L \): change in length; P: pigment; D: complete darkness.
A. Dark-grown State
6 days darkness experimental treatment 24 hr darkness total leaf length measured

B. R-insensitive State
30 min red
5 days darkness 24 hr darkness 24 hr experimental treatment leaves marked (2 mm) Δ length of marks measured

C. White-light-grown State
6 days white light 24 hr experimental treatment leaves marked Δ length measured

Fig. 1. Three conditions under which pea seedlings were grown and the experimental periods employed. Plants grown under these three conditions responded quite differently to light and dark given during the experimental period.

Fig. 2. Growth rates of dark-grown plants exposed to continuous W dim R (20 μw/cm²), or darkness. Total leaf length was measured over a 40-hr period at 4-hr intervals.

response. Or lack of response might be one of the wide range of physiological changes mediated by phytochrome (8).

Red Light-insensitive State. Though not affected by dim R, R-insensitive seedlings still respond to other wavelengths of light. The action spectrum for leaf expansion in R-insensitive seedlings has a major peak in the B region of the spectrum (440 nm) and a minor peak in the O region (630 nm) (Fig. 3). This action spectrum closely resembles the absorption spectrum of protochlorophyll (2). The major absorption peak of protochlorophyll is at 440 nm, but its minor peak can vary between 627 and 648 nm, depending upon the strength of bonding between the pigment molecule and its protein (14). The role of protochlorophyll in chloroplast development and the development of the photosynthetic apparatus (2) suggests that it might act indirectly in controlling leaf expansion.

After R-insensitive seedlings are exposed to W, the B (440 nm) promotion of leaf expansion decreases. As the white-light pretreatment is increased from 0 to 2 hr, response to a subsequent 24-hr exposure to B steadily decreases to half the initial value (Fig. 4). This indicates that protochlorophyll is no longer involved.

Fig. 3. Action spectrum for promotion of leaf expansion in R-insensitive plants. Dark-grown plants were pretreated with 12 hr R and 12 hr darkness to produce the R-insensitive state. Then a 2-mm section was marked on the leaf with India ink and the change in length was measured after a 12-hr exposure to several intensities of monochromatic light. The resulting series of dose-response curves for each wavelength of monochromatic light were linear and parallel to one another. An action spectrum for 20% promotion above dark control was obtained from these dose-response curves.

Fig. 4. Effect of W pretreatment (0-2 hr) on subsequent growth during a 24-hr exposure to B. The W pretreatment was given to plants already pretreated with R (12 hr red—12 hr darkness). White control: R-pretreated plants were exposed to 24 hr of W; dark control: R-pretreated plants were exposed to 24 hr of darkness.
White Light-grown State. This series of experiments employed plants grown entirely under W. White light still promotes leaf expansion ($\Delta L = 2.37$ mm), but no wavelength promotes more effectively than darkness ($\Delta L = 0.82$, 0.85, 0.88, and 0.81 mm for D, G, Y, O, and R). Interestingly, B alone inhibits leaf expansion ($\Delta L = 0.55$ mm).

Because no single wavelength of light promotes leaf expansion, possibly a combination of wavelengths is required. When combinations were tested, only that of B and Y resulted in significant promotion ($\Delta L = 1.58$ mm, compared with 0.92 mm for D and 1.65 mm for W). The B-absorbing receptor, when activated alone, inhibits expansion, but when activated with the Y-absorbing pigment, it helps promote expansion.

The B- and Y-absorbing systems appear to belong to a class of photoreactions known as HER. These require large doses of light for extended periods of time (12). Dose-response curves preliminary to determining action spectra for these two light responses indicate that the Y-absorbing system requires about $10^{22}$ photons/cm$^2$ over a 12-hr period and the B-absorbing system requires a somewhat smaller dose. Both require continuous exposure for promotion. There are examples of HERs with single absorption peaks in the B (4, 10) or in the Y (1) region of the spectrum. Promotion of leaf expansion and inhibition of stem elongation (6) apparently represent the first cases of two HERs which must be activated together for a photosensory. No speculation on the nature of the pigments responsible for these HERs is possible. Since the action peaks of the two photosystems each correspond approximately to one of the absorption peaks of Chl, screening might exert a profound effect on observed action spectra.

The results suggest that there is a transition from one photosensitive state to another in light-controlled leaf expansion in peas: etiolated state (PFr promoted) $\rightarrow$ R-insensitive state (P440, 630 promoted) $\rightarrow$ W-grown state (Pb and Py both required for promotion). Leaf expansion in completely dark-grown plants is phytochrome-mediated. The transition to the R-insensitive state is also phytochrome-mediated. Leaf expansion in the R-insensitive state is probably promoted by protochlorophyll. Expansion in the W-grown state is promoted by two HERs that must be activated in concert. The photosensitive states promoting leaf expansion correspond to those inhibiting stem elongation, indicating coordination of shoot growth in each state.

The photosensitive states coordinating shoot growth as demonstrated in peas might play a role in overtopping, a process in which a plant elongates above competitors and expands its leaves. Competition for light is particularly intense at the seedling stage (7). Shoot coordination in plants germinating in darkness is probably phytochrome-controlled. After a seedling grows above ground but is in low intensity light due to competition, control might be transferred to protochlorophyll. When a plant succeeds in reaching light of fairly high intensity, final control might switch to the two high energy B- and Y-absorbing systems.

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LITERATURE CITED