Comparison of Fluence-Response Relationships of Phototropism in Light- and Dark-Grown Buckwheat

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ABSTRACT

Fluence-response relationships of phototropism in light- and dark-grown buckwheat (Fagopyrum esculentum Moench.) were compared using systematically varied fluence rates and irradiation times of unilateral monochromatic blue light. Etiolated seedlings respond to most fluence rates in a triphasic manner. Phase one differs from classic first positive in that reciprocity is not observed and the peak occurs at a wide variety of fluences, often orders of magnitude less than those characteristic of first positive. Light-grown plants display this pattern only when stimulated by low fluence rates. Phase three is an ascending arm directly related to irradiance time and is comparable to classic second positive. Phase two is a nearly indifferent zone separating phases one and three. At the lowest fluence rates, the maximal observed curvature is greater for dark-grown than for light-grown plants and the former curve more in response to short (2-second) exposures than do the latter. At the highest fluence rates, the maximal observed curvature is much greater for light-grown than for dark-grown seedlings, particularly at irradiation times of 2 to 3 minutes or more. Tropic curvatures correlate positively with increasing fluence rate up to some inflection range, above which the relationship becomes negative. This inflection range is approximately two orders of magnitude higher for light-grown plants.

Following the early studies of phototropism by Darwin (4) and Pringsheim (14) in which a variety of flowering plants and growth conditions were employed, research focused on the response patterns of etiolated oat coleoptiles and Phycocyanes sporangiospores for many decades (5, 13). A major concern in much of this work was the relationship of curvature to the fluence of unilateral light applied. In the most rigorous of these studies, monochromatic blue light at a variety of fluence rates and exposure times was used. Recently, this type of study has been extended to dicots (2, 6, 15). As a result, useful comparisons can be made between the responses of dicot and monocot plants and unifying hypotheses generated (13).

It would likewise be of great interest to compare the sensitivity of light-grown plants to unilateral light with that of dark-grown seedlings. Unfortunately, the vast majority of rigorous studies have employed only one kind (usually complete darkness) of growth environment for the experimental seedlings. In those few studies in which the species investigated was grown in a variety of conditions, the value of the comparisons made is restricted by the use, in each case, of only one fluence rate or, at most, an extremely limited range (1, 3, 7, 8, 10, 12). Further, these rates ranged from 7 (1) to 8000 nmol m^{-2} s^{-1} (10) and the conclusions about the relative phototropic sensitivity of light- and dark-grown plants varied equally widely. One obvious explanation for these conflicting findings is that high fluence rates of unilateral light discriminate between light- and dark-grown plants differently than do low fluence rates. Pringsheim (14) found that light-grown plants curve more than do etiolated ones if high-intensity unidirectional light was tested while the reverse is true if 'weaker' light intensities were tested. This observation should be regarded as suggestive only since unfiltered white light of undefined spectral and energy output was used.

The purpose of the study reported here is to compare the phototropic responses of light-grown and dark-grown buckwheat using monochromatic blue light. The fluence rate was varied over six orders of magnitude and irradiation times ranged from 0.2 to 2700 s. A portion of the data presented for light-grown seedlings has been published previously (6). These findings were extended here through the use of additional fluence rates and shorter exposure times.

MATERIALS AND METHODS

Buckwheat (Fagopyrum esculentum Moench) seeds were obtained from Carolina Biological Supply House, Burlington, NC. Certain details of planting, darkrooms, and experimental procedure have been published elsewhere (6). Planted seeds were placed in a Controlled Environments growth chamber at 25°C under 0.5 W m^{-2} generated by four 40 W GE deluxe cool-white fluorescent and two 35 W incandescent bulbs filtered through 3 to 4 layers of cheesecloth on a 12 h light:12 h dark cycle. Plants designated as 'light-grown' were kept in these conditions.

Plants designated as 'dark-grown' were transferred after 36 h into a light-tight compartment in a photographic dark room at 25°C. Hypocotyls had not yet emerged. Watering was carried out under green light provided by a flashlight covered with Roscolene celluloid (874 + 877). The flashlight is positioned so that the green light does not exceed 0.1 μW cm^{-2} around the plants. Preliminary experiments using high (200 nmol m^{-2} s^{-1}) or low (0.02 nmol m^{-2} s^{-1}) fluence rates of unilateral light indicated that the phototropic behavior of seedlings dark-grown in this manner did not significantly differ from that of seedlings maintained in complete darkness following imbibition. The procedure described was employed because it enhanced seed germination and was more convenient compared with constant darkness. Both light-grown and dark-grown plants were 2 to 3 cm tall and 4 to 5 d old at the time of experimentation.

Unilateral blue light was applied by a Kodak 4000 slide projector mounted in an enclosing black box in conjunction with a 450 nm interference filter (9 nm half-band width; Microcoatings, Inc., Burlington, MA or Oriel Corp.). Fluence rates ranged from 0.003 to 8000 nmol m^{-2} s^{-1} and were obtained through the use of neutral density filters (PTR Optics, Waltham, MA) and varied distance was needed. Light intensities were determined using an International Light 784 Spectroradiometer.

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Ellis System (International Light Inc., Newburyport, MA). Exposure times of 0.2 to 2700 s were used. Longer irradiances were avoided since tonic effects on the sensitivity of dark-grown plants can be observed with prolonged exposures (data not presented). Following the experimental irradiation, plants were incubated in darkness for 75 to 90 min to allow curvature to develop to its maximum.

Each point is the average of five or six separate experiments, each using 8 to 12 seedlings. Standard errors are not shown for the sake of clarity; they ranged from less than ±1.0° for small curvatures (4–20°) to ±2.5° for curvatures greater than 60°.

RESULTS

The phototropic responses of light- and dark-grown buckwheat to different fluences are presented (Fig. 1). Many, but not all, fluence rates induce a tri-phasic pattern. Phase one consists of a response curve which is initiated by irradiance times of 1 s or less, peaks at 2 to 20 s, and shows declining curvature at exposure times of from 20 to 200 s. For example, at a fluence rate of 0.02 nmol m⁻² s⁻¹, phase one begins at less than 10⁻² nmol m⁻² (0.5 s), appears to peak at 4 × 10⁻² nmol m⁻² (2 s), and virtually disappears at 4 × 10⁻¹ nmol m⁻² (20 s) in etiolated plants. It is not comparable to the classic first positive curvature described in other species since: (a) Reciprocity, characteristic of first positive curvature (2, 5, 13, 15, 16), is not observed; instead, the threshold, peak, etc., are strictly time dependent. (b) The fluence at which phase one curvature peaks varies from 0.006 to 4000 nmol m⁻² in dark-grown buckwheat. In other systems, first positive curvature peaks at 100 to 1000 nmol m⁻² (1, 2, 11, 15, 17); phase one in buckwheat can peak at a fluence five orders of magnitude less. Phase three is an ascending arm in which curvature is directly related to irradiance time (or fluence) for any particular fluence rate and is therefore comparable to classic second positive response. Maximum curvature in phase three is almost always greater than that in phase one. Phase two is the nearly indifferent zone between phases one and three. This tri-phase pattern occurs in dark-grown plants in response to all fluence rates but 200 nmol m⁻² s⁻¹; in contrast, only the two lowest fluence rates elicit this pattern in light-grown seedlings. The latter respond to moderate or high fluence rates with only an extended phase two and a phase three.

At the two lowest fluence rates (Fig. 1A), the maximum curvature observed (phase three) is greater in etiolated than in light-grown plants. For example, a photon fluence rate of 0.02 nmol m⁻² s⁻¹ stimulates curvatures of 25° and 11° (P < 0.01) in dark- and light-grown plants, respectively. Further, dark-grown plants are more responsive to short 2 s (= 0.04 nmol m⁻²) exposures. At this fluence rate, dark-grown plants curve 15°, light-grown bend only 6° (P < 0.05). Phase one peak curvatures are not significantly different in the two kinds of plants.

The effects of moderate or intermediate fluence rates are shown in Figure 1B. The lower fluence rate (0.3 nmol m⁻² s⁻¹) induces much greater curvatures in dark-grown plants at both 2 s (0.6 nmol m⁻²) and 20 s (6 nmol m⁻²) irradiance times (P < 0.05); dark- and light-grown seedlings respond very similarly to longer stimuli of unilateral blue light. In contrast, the higher fluence rate (4 nmol m⁻² s⁻¹) causes very similar curvatures of light- and dark-grown plants if short irradiances are used. Longer exposure times induce far greater tropic bending of light-grown and dark-grown plants.

The difference observed at 4 nmol m⁻² s⁻¹ is also seen, although far larger, at the two highest fluence rates thoroughly tested (Fig. 1C). Light-grown plants bend significantly more than etiolated seedlings do. Curvatures of almost 90° are induced by fluence rates of either 200 or 2000 nmol m⁻² s⁻¹ at irradiance times of 6 × 10⁵ m⁻². In contrast, mean curvatures of dark-grown plants do not exceed 20°, even when unilaterally stimulated for 45 min (5 × 10²°; 5 × 10⁵° m⁻²).

Reciprocity was not observed in dark-grown plants at the fluence rates and irradiation times tested. The time dependence of phases one and three is discussed above; similarly, phase two occurs at different fluence levels when plants are given different fluence rates. In light-grown plants, fluences from 10 to 500 nmol m⁻² induce curvatures of 4 to 8° independent of fluence rate between 0.3 and 200 nmol m⁻² s⁻¹. While this might suggest reciprocity, this response level is quite low and flat and could indicate nothing more than a valley of little sensitivity to this fluence range. Nothing observed here corresponds to the response curves with reciprocity in both ascending and descending arms reported for pea (2) and Arabidopsis (15).

The relationships of curvature to fluence rate are presented in Figure 2. This figure includes both data arranged differently in Figure 1 and the results of experiments using additional fluence rates for the exposure times indicated. Resulting curves are qualitatively similar for light- and dark-grown plants but quantitatively different. Tropic curvatures correlate positively with increasing fluence rates up to some inflection range, above which the relationship becomes negative. The inflection fluence rate is approximately two orders of magnitude higher for light-grown plants (about 200 nmol m⁻² s⁻¹) than for dark-grown (about 2.5 nmol m⁻² s⁻¹).

DISCUSSION

Comparison with Tropic Responses of Other Genera. It is generally accepted that the dose-response relationships of phototropism are essentially similar in dark-grown plants, including both monocots and dicots (2, 5, 13, 15). The typical curve resulting from such experiments displays the classic first and second positive responses with an intervening region of low, no, or even negative curvatures. Etiolated buckwheat is an exception to this pattern as phase one differs significantly from first positive.
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Fig. 2. Relationships of curvature and fluence rate for dark-grown (A) and light-grown plants (B). Note the different scales on the Y-axis. Irradiance times are indicated directly on the curves.

Other variations from the common theme have also been recently reported for Arabidopsis (15) and Pisum (2). In the former, classic first positive occurs only in response to certain, intermediate fluence rates; lower and higher rates induce responses which are time-dependent. In the latter, as in corn coleoptiles (11), no second positive occurs. Barley, like etiolated buckwheat, does not possess a phase in which reciprocity is valid (1).

It has been proposed (7, 13) that light-grown plants have lost the high sensitivity system present in etiolated seedlings and thus cannot respond to low, first positive, fluences. However, light-grown buckwheat, stimulated by low fluence rates and short irradiation times (Fig. 1A), curve as much as 12° at fluence levels several orders of magnitude below those normally associated with first positive. These plants, then, are obviously capable of highly sensitive phototropic perception.

Dark-grown seedlings of Arabidopsis (15), pea (2), radish (7), corn (11), and oats (1, 17) have a minimum threshold of from 1 to 10 nmol m⁻² below which curvature does not occur. This same threshold appears at about 10⁻³ and nmol m⁻² in etiolated buckwheat. Since the fluence rates and, in some cases, the irradiance times employed here are significantly less than those used in other studies, it is possible that the difference in observed threshold is more apparent than real and that similar stimulus parameters would yield similar thresholds.

Comparison of Light- and Dark-Grown Buckwheat. The results reported here confirm the early studies of Pringsheim (14); etiolated seedlings curve more than do light-grown plants in response to low fluence rates of unilateral light while the reverse is true at high fluence rates. This helps to explain the wide variation in the conclusions of other studies comparing light- and dark-grown plants. In cases where light-grown or de-etiolated plants were found to be more responsive, fluence rates of 2000 to 8000 (8, 10), 200 (12), or 300 (3) nmol m⁻² s⁻¹ were used. These rates which also induce a much greater curvature of light-grown buckwheat. The same conclusion was reached for barley at substantially lower fluence rates, 4 to 8 nmol m⁻² s⁻¹ (1). In contrast, dark-grown radish curve more than light-grown plants at a fluence rate of 20 nmol m⁻² s⁻¹ (7). These latter two rates fall in a range where the tropic responses of light- and dark-grown buckwheat do not differ dramatically or follow any particular pattern (Fig. 1B). It is not surprising, therefore, given the expected differences in sensitivity between genera, to find some variance in the relative responsiveness of light- and dark-grown seedlings to the fluence rates used for barley and radish. No other study has employed the low rates tested here (Fig. 1A).

As shown in Figure 2, there is a negative correlation between phototropic bending and fluence rate above a certain inflection or transition range, which is almost two orders of magnitude higher for light-grown plants. This pattern was also observed by Pringsheim (14). A possible explanation for the declining response is that at or near the inflection range, photoreceptors on the proximal, but not the distal, side are saturated. Above this fluence rate range, the distal receptors would be increasingly excited, while those on the proximal side, being saturated, would not and the difference between the two would decrease. To the extent the degree of curvature is dependent on the size of this difference, curvature would decrease. The difference between light- and dark-grown plants is not due to the presence of more receptors in the former. If this were true, the threshold fluence rate for phototropism should be significantly lower for light-grown plants; it is not (Fig. 1A).

The simplest explanation for the differences between light- and dark-grown plants at both the lowest and the inflection range fluence rates is that a much greater concentration of masking pigments, such as carotenoids, is present in light-grown tissue (1, 9). The overlap in absorption spectra between the masking molecules and the photoreceptors would decrease the flux of photons in the effective wavelength range available to the receptors mediating phototropism. As a consequence of the different degree of masking in the two kinds of plants, any fluence rate would provide fewer photons for absorption by the receptors for tropism in light-grown plants than in dark-grown. Thus, light-grown plants would curve less than dark-grown at low fluence rates (where no saturation effects occur) and would require a higher fluence rate for saturation of the proximal side photoreceptors (inflection range). Several other explanations are equally plausible. For example, light might induce the synthesis of a second photoreceptor system which is active primarily at high fluence rates but which might partly block perception at very low rates (18).

In these studies, maximal curvature of light-grown plants was much greater than that observed for etiolated seedlings. This difference may be due to the experimental protocol as curvatures of 90° have been induced in dark-grown Arabidopsis through the use of pulsed first positive stimuli (15).

LITERATURE CITED

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