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Characterization of Adaptation in Phototropism of *Arabidopsis thaliana*[1]

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ABSTRACT

Phototropic curvature has been measured for etiolated *Arabidopsis thaliana* seedlings with and without a preirradiation. A bilateral preirradiation with 450-nm light at a fluence greater than about 0.1 micromole per square meter causes a rapid desensitization to a subsequent 450-nanometer unilateral irradiation at 0.5 micromole per square meter. Following a refractory period, the capacity to respond phototropically recovers to the predesensitization level, and the response is then enhanced. The length of the refractory period is between 10 and 20 minutes. Both the time needed for recovery and the extent of enhancement increase with increasing fluence of the bilateral preirradiation. Based on the relative spectral sensitivities of desensitization and enhancement, these responses can be separated. Desensitization is induced by blue light but not by red light. Enhancement, however, is induced by both blue and red light. Thus, enhancement can be induced without desensitization but not vice versa. Both desensitization and enhancement affect only the magnitude of the response and do not affect the fluence threshold.

It has long been known that receptor systems in plants have a built-in process for producing a change in sensitivity to a stimulus. In this process, known as adaptation, an exposure to a stimulus may be followed by a refractory period, during which the plant is insensitive to the stimulus. After the refractory period, full sensitivity is slowly regenerated (7). Adaptation also occurs in phototropism (1, 3, 4, 5, 7). This poses particular problems for the study of second positive phototropism, because relatively long exposures to light are required, and the sensitivity of the plant may vary during the course of an exposure.

The objective of this work was to characterize adaptation in phototropism by *Arabidopsis thaliana* as a first step toward evaluating adaptation's role in second positive phototropism. We show that adaptation consists of desensitization, a refractory period, recovery, and enhancement. The first three occur following an exposure to blue light while enhancement occurs following an exposure to either blue or red light.

MATERIALS AND METHODS

Seedlings of *Arabidopsis thaliana* (L.) Heynh. strain 'Estland' were grown as previously described (6) in strips of microassay wells containing 0.7% (w/v) agar. Seed germination was potentiated by chilling at 5 ± 1°C in darkness for 3 d, and then exposing to white light for 20 h at 25 ± 1°C. Following the white light irradiation, the strips were transferred into darkness at 25 ± 1°C for 42 h, at the end of which the seedlings were exposed to the appropriate photostimulus. The seedlings were maintained throughout at a RH greater than 90%. Because green light is known not to be phototropically 'safe' (9), all manipulations were performed in complete darkness.

Light Sources

The white light (50 μmol m^{-2} s^{-1}), which was used to potentiate seed germination, was provided by two General Electric (Cleveland, OH) Delux Cool-White fluorescent tubes. A slide projector equipped with a Sylvania 900 W BVA tungsten-halogen lamp (Danvers, MA), in combination with the appropriate Corion (Holliston, MA) interference filter (10-nm half-band width; stray light blocked to >2000 nm) was used as the light source in the phototropism experiments. The preirradiation was given either from above or from two opposing sides. The latter bilateral irradiation was accomplished by sequentially irradiating opposite sides of the seedlings for equal times and equal fluence rates. The preirradiation was followed after some time period by a unilateral irradiation at 450 nm to induce phototropism. Fluence rates were measured using a Li-Cor (Lincoln, NE) LI-190SA quantum sensor in combination with a LI-1000 Datalogger. The duration of irradiation was controlled with a Uniblitz (Vincent Associates, Rochester, NY) shutter.

There are difficulties with either a preirradiation from above or a bilateral preirradiation. The hook may optically shade the photoperceptive zone if the plant is irradiated from above. For this reason and because similar results were obtained using both preirradiations, the bilateral preirradiation was used in all subsequent work. However, it is technically impossible to ensure precisely the same fluence rate on opposite sides of the seedling. To overcome this limitation, the bilateral preirradiation was administered as two sequential irradiations using the same light beam, and rotating the seedling by 180° between the irradiations. This is referred to as a single preirradiation. The required bilateral fluences were obtained by varying the fluence rate and times of irradiation.

Measurement of Curvature

Experiments were terminated 60 min after the end of the last light stimulus as previously described (6). The seedlings

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were then gently mounted on transparent adhesive tape with the direction of bending in the plane of the tape surface. The tape was inserted into a photographic enlarger and the hypocotyl curvature traced. Only seedlings that emerged upright (within a solid angle of ±10°) from the agar were used. Curvature was measured as previously described (8).

RESULTS

Assay for Adaptation

A single unilateral flash of 450-nm light at 0.5 μmol m^{-2} induces a curvature of 11.6 ± 1° in etiolated *Arabidopsis* seedlings (Table I). However, if the seedlings are first exposed to 450-nm light from above at 20 μmol m^{-2}, and then exposed, within 2 s, to the unilateral flash, the seedlings develop little, if any, curvature. If the time between the first irradiation (from above) and the second irradiation (unilateral) is increased to 20 min, the seedlings subsequently develop curvature of about 10°. Thus, the elements of adaptation can be seen in *Arabidopsis*. The seedlings change their responsiveness following an exposure to light and, after some refractory period, recover their original responsiveness.

The decrease in sensitivity to a unilateral irradiation induced by a preirradiation appears to be a loss of response rather than a shift in the fluence required for phototropism (Fig. 1). The fluence-response curve for phototropism shows no response at any fluence from 0.01 to 15 μmol m^{-2} immediately following a preirradiation either from above (Fig. 1A) or bilaterally (Fig. 1B). Moreover, as the response reappears following either preirradiation, only the magnitude of the response varies; neither the threshold nor the optimum fluence changes (Fig. 1).

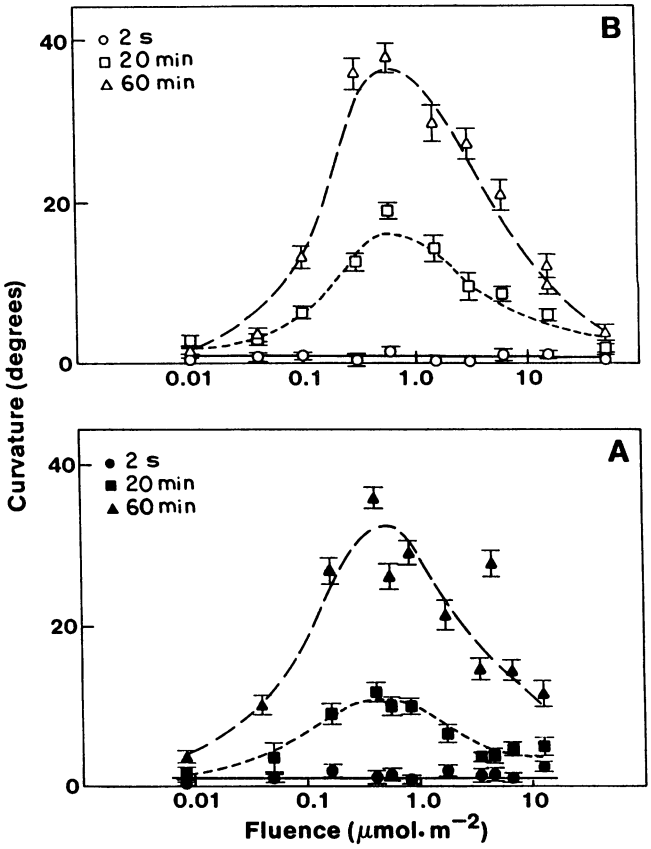

Figure 1. Fluence-response relationships for phototropism following a desensitizing preirradiation. Etiolated seedlings were irradiated from above (A, closed symbols) or bilaterally (B, open symbols) with (450-nm light at 20 μmol m^{-2}, and then irradiated unilaterally after a dark interval of 2 s (circle), 20 min (square), or 60 min (triangle) with 450-nm light at 0.24 μmol $m^{-2}s^{-1}$ at appropriate times to give the fluence indicated on the abscissa. Curvature was measured 60 min after the last irradiation. Each data point represents the mean curvature of 90 to 110 seedlings ±1 SE.

Fluence Dependence of Adaptation

To define further the dependence of adaptation on fluence of the preirradiation, seedlings were bilaterally preirradiated at different fluences and then unilaterally irradiated at 0.5 μmol m^{-2} at different times from 5 to 120 min following the preirradiation. The results show no measurable suppression of curvature by a bilateral preirradiation of less than about 0.2 μmol m^{-2} (Fig. 2A). However, hypocotyl curvature gradually decreased as the preirradiation fluence, given 5 to 10 min before the unilateral irradiation, increased above about 0.2 μmol m^{-2}. Little or no curvature was obtained when the preirradiation fluence, applied 5 to 10 min prior to the unilateral irradiation, was higher than 1 μmol m^{-2}.

Seedling responsiveness to the unilateral irradiation is regained, following a refractory period of between 10 and 20 min, and is then enhanced. The recovery and enhancement of responsiveness is a function of preirradiation fluence and time in darkness between the preirradiation and the unilateral irradiation (Fig. 2, A and B). This is shown more directly by plotting the data from Figure 2 as a function of the time interval between the bilateral preirradiation and the unilateral irradiation (Fig. 3). Responsiveness to the unilateral irradiation is regained with time following the refractory period. The time needed to regain the initial level of responsiveness (*ca.* 10° curvature) is between 10 and 20 min for bilateral irradiations of 23.6 μmol m^{-2} or less, but at fluences above 23.6 μmol m^{-2}, it is greater than 20 min. In addition, the final curvature measured at the end of the experiment is generally higher for the higher fluences of the bilateral irradiation but decreases at very high fluences (>100 μmol m^{-2}).

Table I. *Dependence of Phototropic Curvature in Arabidopsis on a Preirradiation of Seedlings*

Preirradiation[a] (from above)	Unilateral Irradiation[a]	Time between Pre- and Unilateral Irradiations	Curvature
μmol m^{-2}			degrees ± SE[b]
0	0.5	0	11.6 ± 1.0
20	0.5	2 s	1.4 ± 0.7
20	0.5	20 min	10.0 ± 1.1

[a] Single flash of 450-nm light. [b] The means represent the average curvature of at least 100 seedlings. Curvature was measured 60 min after the end of the unilateral irradiation.

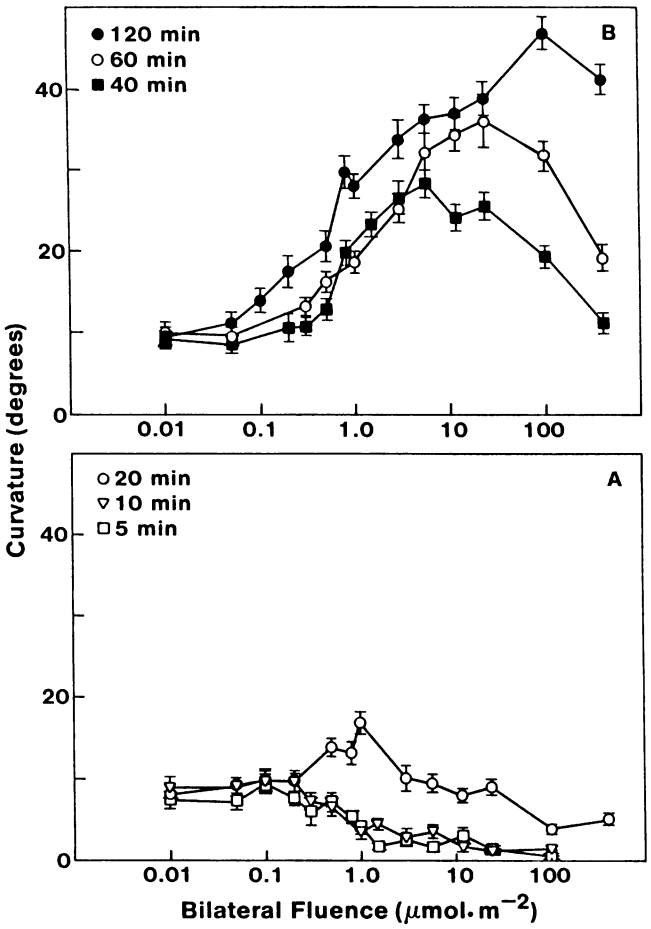

Figure 2. Fluence-response relationships for adaptation of phototropism in *Arabidopsis*. Etiolated seedlings were irradiated bilaterally with 450-nm light at the indicated fluences, and then irradiated unilaterally after a dark interval of (A) 5, 10, or 20 min; (B) 40, 60, or 120 min with 450-nm light at 0.5 μmol m^{-2}. Curvature was measured 60 min after the last irradiation. Each data point represents the mean curvature of 90 to 110 seedlings ±1 SE.

Spectral Sensitivity for Desensitization and Enhancement of Phototropism

Preliminary experiments (data not shown) indicated that preirradiation with some wavelengths of light cause an enhancement of the phototropic response without first causing desensitization. More detailed experiments, using bilateral preirradiations with different wavelengths of light, indicated that desensitization was induced by blue light, and to a lesser extent by green light, but not by red light (Table II). In contrast, the enhancement of phototropism was induced by both blue and red light, but not by low wavelength green light (Table III).

DISCUSSION

Based on these results, the process of adaptation in *Arabidopsis* consists of several steps. The first step is desensitization in which an exposure to light causes an abrupt decrease in the phototropic responsiveness of the plant to a subsequent light exposure given within 1 min of the preirradiation (Table I, Fig. 1). This decrease in responsiveness appears not to be a shift in sensitivity to light since the fluence response curve appears not to be shifted. Rather, desensitization appears to affect only the capacity for curvature. Second, following desensitization, the plants go through a refractory period during which they are insensitive to a phototropic stimulus. The duration of the refractory period is between 10 and 20 min (Fig. 3). Third, after the refractory period, phototropic responsiveness recovers, and the rate of recovery is dependent on the preirradiation fluence, increasing with increasing fluence (Fig. 3). The longer times required for recovery and enhancement following high fluences of preirradiation may explain the apparent decrease in curvature following these higher fluences (Fig. 2B).

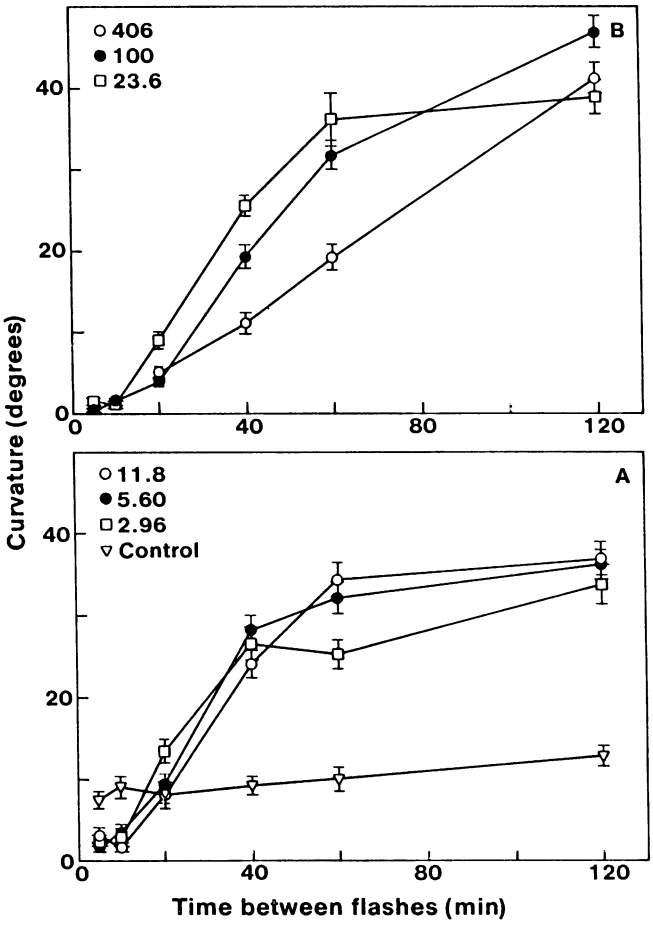

Figure 3. Kinetics of recovery and enhancement of the capacity for phototropism following desensitization in *Arabidopsis*. Seedlings were irradiated bilaterally with 450-nm light at fluences of 2.96 to 406 μmol m^{-2}, and then irradiated unilaterally with 450-nm light at 0.5 μmol m^{-2} at the indicated times following the bilateral irradiation. Curvature was measured 60 min after the last irradiation. Data points represent the mean curvature of 90 to 110 seedlings ±1 SE. (A) Fluence-response curves for bilateral irradiations of 0, 2.96, 5.6, and 11.8 μmol m^{-2}. (B) Fluence-response curves for bilateral irradiations of 23.6, 100, and 406 μmol m^{-2}. The control curve represents the curvature of seedlings which received the 0.5 μm m^{-2} unilateral irradiation but no bilateral irradiation.

Table II. *Wavelength Dependence for Desensitization*

Etiolated seedlings were preirradiated bilaterally with 15 μmol m^{-2} of light at the indicated wavelength, and then, within 1 min, irradiated unilaterally with 0.5 μmol m^{-2} of 450-nm light.

Preirradiation Wavelength	Curvature
nm	*degrees ± SE*[a]
None	11.6 ± 1.0
405	0.2 ± 0.3
428	1.4 ± 1.4
450	0.4 ± 0.4
478	0.3 ± 0.5
510	6.5 ± 1.2
560	8.2 ± 0.8
601	9.8 ± 0.8
640	11.2 ± 1.0
669	10.5 ± 1.1

[a] The means represent the average curvature of at least 100 seedlings. Curvature was measured 60 min after the end of the unilateral irradiation.

The fourth step is an enhancement of phototropic responsiveness, such that the angles of curvature obtained are considerably greater than those induced by the same fluence without the preirradiation. The extent of enhancement depends on the fluence of preirradiation, and on the dark interval between the preirradiation and the unilateral irradiation. Thus, the maximum curvature is obtained with increasingly higher fluences and longer dark intervals. The phenomena of desensitization and recovery observed here are similar to those previously reported (1, 2, 4, 5). In corn coleoptiles, enhancement is not seen, and in addition, recovery is incomplete (4). In contrast, we observe a distinct enhancement of curvature following complete recovery. Therefore, enhancement may be organ or plant specific.

Phototropic curvature in response to a given fluence of light in *Arabidopsis* is known to be increased by giving that fluence in a series of flashes, separated by periods of darkness of 15 to 20 min (8). This pulse effect has been interpreted to result from a kinetic limitation in the transduction sequence restricting the magnitude of phototropic curvature. The kinetic limitation has been attributed to some rate-limiting step after the photoreceptor pigment and not to a limitation in the absorption of quanta. Thus, the pulse effect appears not to be the same as the enhancement reported here, since the pulse effect is optimal at time intervals of 20 min (8), while enhancement increases with time up to 120 min following the bilateral irradiation.

Both desensitization and enhancement in *Arabidopsis* involve a modulation of the magnitude of the phototropic curvature. Neither process affects either the fluence threshold for first positive phototropism or the fluence for maximal first positive curvature. In contrast, the desensitization described by Iino (4, 5), involves a shift in the entire fluence-response curve for phototropism. We do not understand the difference between these two descriptions of desensitization. They could result from the use of different species, or from the use by Iino (5) of corn which has been preirradiated with red light, which is known to affect subsequent phototropism (Table III) (5). Galland (3) described two types of adaptation. In one type, sensor adaptation, the range of the sensor is modulated. The adaptation described by Iino (4, 5) is of this first type. The second type is effector adaptation, in which the output of the transduction chain is modulated. The adaptation in *Arabidopsis*, which is described here, is clearly effector adaptation and not sensor adaptation. Thus, we conclude that adaptation in etiolated seedlings of *Arabidopsis* is not a modulation of the step or steps that set the threshold fluence for phototropism.

We have shown that second positive phototropism exhibits two thresholds (6). First, there is a threshold in number of quanta which is the same as the threshold for first positive phototropism. Second, there is a threshold in time of irradiation. Second positive curvature is observed when both thresholds are exceeded. Thus, relatively long irradiation times are required for second positive curvature although the length of time may be species specific (*cf.* ref. 4). Therefore, adaptation must be an element in any model for phototropism.There is no evidence at present to indicate whether or not the photoreceptor pigments for phototropism are involved in the control of adaptation in *Arabidopsis*. Based on the data of Iino (5), both phytochrome and a blue light system regulate adaptation in *Zea*. Although there are no data to indicate whether enhancement in *Arabidopsis* is distinct from recovery or is simply an extension of the same process, it is certainly possible that enhancement is the same as the slow, phytochrome-mediated response in *Zea* (5). The relative spectral sensitivity for enhancement (Table III) is consistent with this view. However, it is also clear that desensitization is not phytochrome-mediated. Action spectra for the steps in adaptation, combined with the use of mutants altered in one or more components, should permit an analysis of the regulating pigments.

Zimmerman and Briggs (10, 11) suggested that separate systems function in first positive and second positive phototropism. Based on the data presented by Janoudi and Poff

Table III. *Wavelength Dependence for Enhancement*

Etiolated seedlings were preirradiated bilaterally with 3.5 × 10^{-2} μmol m^{-2} of light at the indicated wavelength, left in darkness for 2 h, irradiated unilaterally with 0.5 μmol m^{-2} of 450-nm light.

Preirradiation Wavelength	Curvature
nm	*degrees ± SE*[a]
None	9.5 ± 1.0
428	14.2 ± 1.2
450	16.1 ± 1.2
510	8.5 ± 1.1
531	8.7 ± 1.6
560	16.1 ± 1.5
601	20.4 ± 1.8
640	24.0 ± 1.7
669	27.8 ± 2.1

[a] The means represent the average curvature of at least 100 seedlings. Curvature was measured 60 min after the end of the unilateral irradiation.

(6), there is no reason to believe that these two systems are different photoreceptor pigment systems. Based on the results presented here, we agree with the suggestion of Iino (5) that the difference between first positive and second positive phototropism is the existence of a time threshold for second positive phototropism, and the contribution to second positive phototropism by the slow components (*i.e.* recovery and enhancement) of adaptation. It is clear that an analysis of adaptation must precede an understanding of second positive phototropism.

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