Time Threshold for Second Positive Phototropism Is Decreased by a Preirradiation with Red Light

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ABSTRACT

A second positive phototropic response is exhibited by a plant after the time of irradiation has exceeded a time threshold. The time threshold of dark-grown seedlings is about 15 minutes for Arabidopsis thaliana. This threshold is decreased to about 4 minutes by a 669-nanometer preirradiation. Tobacco (Nicotiana tabacum) seedlings show a similar response. The time threshold of dark-grown seedlings is about 60 minutes for tobacco, and is decreased to about 15 minutes after a preirradiation with either 450- or 669-nanometer light. The existence of a time threshold for second positive phototropism and the dependence of this threshold on the irradiation history of the seedling contribute to the complexity of the fluence response relationship for phototropism.

Understanding the mechanism for phototropism has been hindered by the complexity of the fluence response relationship for this response by seedlings. Phototropic curvature increases with increasing fluence in an ascending arm above a threshold fluence, reaches a maximum, and then decreases in a descending arm. This relatively symmetrical curve is known as first positive phototropism. At even higher fluences, curvature increases again with increasing fluence in what is known as second positive phototropism. Reciprocity is valid for first positive phototropism but not for second positive phototropism (3, 8).

The suggestion by Zimmerman and Briggs (12, 13) that first and second positive phototropism result from separate systems has been interpreted to mean that the two phototropic responses are controlled by different photoreceptor pigments. However, Janoudi and Poff (5) suggested that the apparent lack of valid reciprocity for second positive phototropism is, at least in part, a consequence of an irradiation time requirement for that response. When that time requirement is met in Arabidopsis thaliana, fluence response relationships for second positive phototropism show a fluence threshold that is the same as the fluence threshold for first positive phototropism. Based on this common fluence threshold for first and second positive phototropism, they noted that it is not necessary to postulate a difference in the photoreceptor pigment for the two phototropic responses. Rather, the existence of second positive phototropism and its apparent lack of valid reciprocity follow from the time requirement.

An examination of the literature on phototropism shows apparent fluence thresholds for second positive phototropism corresponding to time thresholds from 3 to 11 min (e.g. 1, 2, 7, 12). Because the amplitude of phototropic curvature to blue light can be increased by a preirradiation with red light, it was reasonable to measure the effect of such a preirradiation on the time threshold for second positive curvature. In this paper, we characterize the time threshold for second positive phototropism by two dicotyledonous plants, A. thaliana (L.) Heynh. and tobacco (Nicotiana tabacum L.). We show that this time threshold is substantially different for the two plants and that the threshold is decreased by a red-light preirradiation.

MATERIALS AND METHODS

Seedlings of the Estland strain of Arabidopsis thaliana and of the Wisconsin 38 cultivar of tobacco (Nicotiana tabacum) were grown in strips of microassay wells containing 0.7% (w/v) agar (5). Seed germination was potentiated by chilling the seeds at 5°C ± 1°C in darkness for 2 to 3 d, and then exposing to white light for 20 h for Arabidopsis and 24 h for tobacco at 25°C ± 1°C. The strips were then transferred into darkness at 25°C ± 1°C for 42 h for Arabidopsis and 5 d for tobacco. At the end of this dark period, the seedlings were exposed to the appropriate phototropic stimulus. The seedlings were maintained throughout at an RH greater than 90%. All manipulations were performed in complete darkness because green light is known not to be phototropically "safe" (10).

Light Sources

White light (50 µmol m⁻² s⁻¹) from two General Electric (Cleveland, OH) Delux Cool-White fluorescent tubes was used to potentiate seed germination. The light source in the preirradiation and phototropism experiments was a slide projector equipped with a Sylvania (Danvers, MA) 900 W tungsten-halogen lamp, in combination with an appropriate Corion (Holliston, MA) interference filter (10-nm half-bandwidth; stray light blocked to >2000 nm).

For both Arabidopsis and tobacco, the red-light preirradiation was given as a bilateral irradiation by sequentially irra-
diating opposite sides of the seedlings with 669-nm light for 65 s at a fluence rate of 0.15 µmol m\(^{-2}\) s\(^{-1}\). The blue-light preirradiation was administered similarly with the use of 450-nm light for 30.3 s at a fluence rate of 0.33 µmol m\(^{-2}\) s\(^{-1}\). The phototropism-inducing irradiation was given unilaterally. Fluence rates were measured using a Li-Cor (Lincoln, NE) LI-190SA quantum sensor in combination with an LI-1000 Datalogger, the duration of irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY).

Measurement of Curvature

The seedlings were removed from darkness 70 min after the end of the last light stimulus and 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 image of hypocotyl curvature was traced. Only seedlings that emerged upright (within a solid angle of 10\(^{\circ}\)) from the agar were used. Curvature was measured as previously described (9).

RESULTS AND DISCUSSION

The time threshold for second positive phototropism by Arabidopsis seedlings was measured by irradiating the seedlings with 30 µmol m\(^{-2}\) of 450-nm light for time periods of 60 to 4000 s. Seedlings that had not been preirradiated with red light exhibited second positive phototropism at times greater than about 10 to 15 min (Fig. 1). However, when the seedlings were preirradiated with red light (669 nm) 120 min before the inductive blue light, this time threshold decreased to about 4 min (Fig. 1).

To check the generality of this red light-induced decrease in the time threshold, phototropism of tobacco was measured. Seedlings were exposed to varying fluences of blue light at 0.33 µmol m\(^{-2}\) s\(^{-1}\). Curvature was permitted to develop for 70 min in darkness and then measured. The fluence response relationship for tobacco seedlings that had not been preirradiated with red light shows first positive phototropism at fluences from about 0.5 to about 5 µmol m\(^{-2}\). Second positive phototropism is observed at fluences > 1000 µmol m\(^{-2}\) for a time threshold of about 60 min (Fig. 2).

The phototropic responsiveness of tobacco seedlings is enhanced by a preirradiation with either 450-nm or 669-nm light at 10 µmol m\(^{-2}\) given 120 min before irradiation with an inductive blue light pulse (Figs. 2 and 3). In addition, the phototropic capacity of tobacco can be eliminated by a desensitizing irradiation with 450-nm light at 10 µmol m\(^{-2}\) (data not shown). After desensitization, recovery and enhancement of phototropic capacity are completed within 120 min. Desensitization, recovery, and enhancement have been demonstrated as components of adaptation in phototropism of Arabidopsis (6). After recovery, the response in tobacco seedlings is enhanced, as in Arabidopsis. This enhancement is also seen if a fluence response relationship is measured 2 h after 669-nm irradiation (Fig. 2). For sensor-type adaptation, one expects the threshold fluence to shift to some higher fluence upon desensitization and then to shift back upon recovery, whereas one expects the threshold fluence not to shift in response-type adaptation (4). On the basis of the data presented in this paper, one cannot identify the type of adaptation in tobacco.

The time threshold for second positive phototropism in tobacco is decreased from the 60-min time threshold for the non-preirradiated seedlings (Figs. 2 and 3) to about 15 min by a preirradiation with either 669- or 450-nm light (Figs. 2 and 3). This blue and red spectral sensitivity for the time threshold shift is similar to that for enhancement (6) and differs from the spectral sensitivity for desensitization, which is a blue-light response (6).

We conclude from the data presented here that the decrease in time threshold upon red-light preirradiation is not limited to Arabidopsis. Fluence response relationships have been de-

![Figure 1. Dependence of phototropism on the time of irradiation for seedlings of A. thaliana. ▲ Non-preirradiated; curvature induced by 30 µmol m\(^{-2}\) of 450-nm light; △, preirradiated with 10 µmol m\(^{-2}\) of 669-nm light 2 h before induction of curvature with 450-nm light. Each point represents the mean curvature of 100 to 180 seedlings. Vertical bars represent ± 1 se.](https://www.plantphysiol.org/)

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scribed previously for a number of species such as Pilobolus crystallinus (7) and Avena sativa (1, 12, 13). The time thresholds for second positive phototropism calculated from these published papers range from about 5 min for Avena (1) to about 11 min for Pilobolus (7). The interspecific variability is perhaps not surprising. However, variability is observed even for a single species, with values for Avena ranging from 5 min (1) to 11 min (12, 13). We suggest that this interspecific variability is a consequence of the different preirradiation conditions. Zimmerman and Briggs (12, 13) presented fluence response curves for dark-grown and red-preirradiated plants. On the basis of the apparent fluence threshold and the fluence rate used, we have calculated a time threshold of about 10 to 11 min for dark-grown seedlings and about 3 to 4 min for seedlings that had been preirradiated with red light. On the basis of the results presented here with Arabidopsis and tobacco, we attribute this difference in time threshold in the data of Zimmerman and Briggs (12) to the red-light preirradiation.

It should not be ignored that the use of “safe” lights could contribute to apparent variations in the time threshold. Phototropism of dark-grown seedlings cannot be considered equivalent to phototropism of red-light preirradiated seedlings because red light affects enhancement and the value for the time threshold of second positive phototropism. Thus, these data support the previous contention that no visible radiation can be considered “safe” for phototropism (10).

Enhancement and the time threshold shift could be mani-

**Figure 2.** Fluence response relationship for phototropism of tobacco seedlings. ▲, Non-preirradiated; curvature induced by 450-nm light; Δ, preirradiated with 10 μmol m⁻² of 660-nm light 2 h before inducing curvature with 450-nm light. Each point represents the mean curvature of 100 to 145 seedlings. Vertical bars represent ± 1 se.

**Figure 3.** Fluence response relationship for phototropism of tobacco seedlings. ▲, Non-preirradiated; curvature induced by 450-nm light; Δ, preirradiated with 10 μmol m⁻² of 450-nm light 2 h before inducing curvature with 450-nm light. Each point represents the mean curvature of 100 to 145 seedlings. Vertical bars represent ± 1 se.
festations of one red-light effect, or they could be completely separate responses. Insufficient data are available to distinguish these two possibilities. Measurements of the fluence dependence for both responses are in progress and may permit such an analysis.

It is reassuring that the mechanism that sets the time threshold is sufficiently plastic that the threshold for tobacco is very different from that of Arabidopsis. Thus, we are reasonably confident that mutants in Arabidopsis can be found in which this threshold is altered. An explanation for the biochemical basis for the time threshold may require the availability of such mutants. In the absence of any data, we conjecture that the ‘timer’ that sets the time threshold is, in fact, a time-dependent biochemical reaction. If this conjecture is correct, we also expect a temperature dependence for that reaction, and thus also for the time threshold itself.

The complexity of the fluence response relationship for phototropism has impeded research on phototropism for many decades. Although there is no basis for concluding that separate photoreceptor pigments regulate the induction of first positive and second positive phototropism, there is clear evidence that different underlying mechanisms operate in these two responses. In particular, reciprocity is valid for first positive phototropism. In contrast, reciprocity is not valid for second positive phototropism at least in part because of the time threshold for that response (5, 6). Moreover, the value for this time threshold itself depends on the irradiation history of the seedling. Thus, the special characteristics of second positive phototropism result from the process of adaptation, proceeding along with the less complex photochemistry of first positive phototropism.

It follows from the dependence of the time threshold on the irradiation history of the seedling that, under field conditions, with long periods of irradiation each day, the time threshold for second positive phototropism should be at a minimum, and consistently exceeded. Although much has been learned about phototropism by emphasizing first positive phototropism for which reciprocity is valid, this emphasis has prevented us from appreciating the complexity of second positive phototropism, which is exhibited under field conditions where reciprocity fails.

LITERATURE CITED