Phytochrome Control of Another Phytochrome-mediated Process

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ABSTRACT

The phytochrome-mediated attachment of root tips of mung bean (Phaseolus aureus) and barley (Hordeum vulgare) to glass is affected by the prior exposure of hydrated seeds or seedlings to red or far red radiation. Prior irradiation of seeds or seedlings of mung bean with red light promotes attachment, while far red light promotes detachment of root tips. Similar exposure of barley seeds and seedlings to red light accentuates detachment, while far red light accentuates attachment of root tips. Red–far red light reversibility of the pretreatments indicates phytochrome control.

Indoleacetic acid concentrations of 10 nM or higher appear to mimic the effects of far red light pretreatments in both mung bean and barley root tips.

One of the earliest processes elicited by phytochrome after red or far red irradiation is probably a subtle alteration in some property of the plasmalemma which could result in changes in permeability (1) or surface electrical potentials. Because of this modification in surface charges, root tips can be made to attach to a glass surface charged with phosphate ions following red irradiation and to detach following far red irradiation (7–9). This photoreversible attachment has been correlated with a red, far red light reversible bioelectric potential change in mung bean root tips (3).

During the development of an improved method to assay the phytochrome-mediated attachment of root tips to glass, mung bean root tips were observed to behave in an erratic manner. This vagary in behavior was minimized by exposing imbied seeds or seedlings to a constant red or far red light source prior to excision of root tips. This paper reports on the method used to determine whether phytochrome was responsible for the erratic behavior of the root tips.

MATERIALS AND METHODS

Mung bean seeds (Phaseolus aureus L., var. Oklahoma 612) and barley seeds (Hordeum vulgare L., var. Compana) were soaked in darkness for 8 hr. They were spread in dim light (0.01 μW cm⁻² at 600–700 nm, 0.02 μW cm⁻² at 700–800 nm) over cheesecloth in a porcelain crock containing deionized water. The hydrated seeds were then exposed to red or far red light before being placed in darkness at 24 C with continuous aeration for 2 (barley) or 3 days (mung bean). Red light was from two 15-w daylight fluorescent tubes filtered with two layers of red cellophane (65 μW cm⁻² from 600–700 nm). The far red light source was a 60-w tungsten filament lamp covered with four sheets of red cellophane, two sheets of blue cellophane, and a filter of 5 cm of water (190 μW cm⁻² from 700–750 nm).

Two or 3 days later, a selected number of seedlings was exposed to red (14.8 μW cm⁻² from 650–680 nm) or far red light (17.3 μW cm⁻² from 710–750 nm) for a few minutes and placed in darkness. Four hours later, 10 root tips of 2 mm length were excised under dim light and washed once with glass-distilled water in a glass beaker. Ten milliliters of the following solution were then added to the beaker: KCl, 0.1 mM; MgCl₂, 0.1 mM; CaCl₂, 0.1 mM; MnCl₂, 2 μM; L-ascorbic acid, 1 μM; and IAA, 0.2 nm. The beaker was placed immediately on a phonograph turntable in a beam of red light (4.0 μW cm⁻² from 650–680 nm). The root tips were centered

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MUNG BEAN (light exposure to seeds: 4' R, 8 FR)

In the beaker about 2.5 cm from the center of the turntable. After 1 min of irradiation, the turntable was gradually accelerated to 33 rpm for a few revolutions. The number of root tips remaining attached was counted while the turntable was gradually slowed down. This procedure was repeated once every minute for 5 min under red light. Following the red light exposure, the tips were handled in the same manner under far red light (8.9 μW cm⁻², 710–750 nm) for 7 min to complete one cycle. Root tips were observed under dim light (0.007 μW cm⁻² from 600–700 nm, 0.012 μW cm⁻² from 700–800 nm) throughout both red and far red light exposure.

The red and far red lights used to irradiate seedlings and root tips were isolated from light radiating from a 35-w projection lamp (Sylvania, type DKN) with filters of 9 cm of water and interference filters (Baird-Atomic, type B-9, peak transmissions at 660 and 730 nm). Irradiance were measured with an interference filter spectroradiometer (courtesy of K. H. Norris).

RESULTS

The photoreversible attachment of root tips is apparently influenced by some process initiated by light in hydrated seeds or seedlings. This can be seen in some results presented in Figures 1 to 4. These kinds of results were obtained in repeated trials extending over several months.

When hydrated seeds of mung beans are exposed to red light and germinated in darkness, the root tips excised from resulting seedlings attach to glass after red irradiation and detach after far red irradiation (Fig. 1a). Root tips from similar seedlings preexposed 4 hr before excision to 4 min of far red, however, show poor attachment in both red and far red light (Fig. 1b). Further results presented in Figure 1, c to e, demonstrate that the photoreversible attachment is influenced by the last light exposure received in the preirradiation treatment. Red light pretreatment promotes strong attachment in red, while far red light pretreatment promotes weak attachment in red. Increasing the concentration of IAA (10 μm) in the assay medium results in poor attachment of root tips from seedlings of seeds exposed to red light (Fig. 1f).

If immediately after red light exposure the hydrated seeds of mung bean are exposed to far red light, the root tips obtained from resulting seedlings show slight adhesion to glass when placed in red light (Fig. 2a). Exposing such seedlings to red light promotes later attachment of their root tips in red light (Fig. 2b). Additional light pretreatments show again that the behavior of root tips is governed by the final light exposure in the pretreatment (Fig. 2, c to e). Once more, little attachment is shown by root tips from red light pretreated seedlings when tips are placed in a solution with an increased concentration of IAA (Fig. 2f).

The effects of light pretreatment on barley root tips are presented in Figures 3 and 4. As in the case of mung bean root tips, the photoreversible attachment of barley root tips on glass is controlled by the last light exposure given to hydrated seeds or seedlings. Unlike mung bean root tips, however, if the last light exposure was red, barley root tips show tendency to detach in far red (Fig. 3, a, c, and e; Fig. 4, b and d); and
BARLEY (light exposure to seeds: 4' R, 8' FR)

Light exposure to seedlings: x-dark (a)

Light exposure to seedlings: x-2' R (b)

Light exposure to seedlings: x-2', 4' FR, 2' R (c)

Light exposure to seedlings: x-2', 4' FR, 2' R (d)

Light exposure to seedlings: x-2', 4' FR, 2' R, 4' FR (e)

NUMBER OF ATTACHED ROOT TIPS

MINUTES OF IRRADIATION

FIG. 4. Effects of prior irradiation of hydrated seeds of barley with far red light after red light and of the resulting seedlings with red and far red light on the photoreversible attachment of root tips to glass. Root tips were excised 4 hr after exposure of seedlings to light. R: red light; RF: far red light.

If the final light exposure was far red, they fail to detach in far red (Fig. 3, b and d; Fig. 4, a, c, and e). Again unlike mung bean root tips, barley root tips show strong attachment in high concentrations of IAA (Figs. 3f and 4f).

DISCUSSION

From the results presented, red and far red light appear to be able to initiate processes which can influence, several hours or days later, the photochrome-mediated attachment of root tips to glass. That this process is likely to be under photochrome control is readily seen by its repeated red, far red reversibility. An indication of the nature of this process is furnished by the rapid effect of IAA at higher concentrations. Like the effect of far red light pretreatment on hydrated seeds and seedlings, "high" concentrations of IAA promote, on the one hand, attachment of barley root tips, and on the other hand, detachment of mung bean root tips. These results suggest that photochrome could control the photoreversible attachment in root tips through a process affecting the levels of IAA and possibly other hormonal substances in root cells. After far red irradiation, the endogenous level of IAA could increase to such an extent that rapid photoreversibility is no longer possible.

The present study offers no clues to the specific mechanism as to how photochrome can control the endogenous level of IAA. Several investigators (2, 5) have shown that the activity of IAA oxidase is influenced by photochrome.

Indications that the levels of other hormones could be affected have been obtained recently. The findings of Reid et al. (6) that red irradiation of barley leaf soon results in higher levels of gibberellic acid suggest a possible photochrome control of GA levels. Jaffe (4) has recently claimed that photochrome controls the endogenous levels of acetylcholine in mung bean root cells.

The difference in behavior between mung bean and barley following red or far red light pretreatment and IAA is difficult to explain. It is possible that the basis for this difference lies in their dissimilar response to a given photoperiod. Further work with more long and short day plants might clarify this question.

LITERATURE CITED