Enhanced Phytochrome Sensitivity and Its Reversal in
Amaranthus albus Seeds

ABSTRACT

Seed of Amaranthus albus L. develop an enhanced sensitivity to the far-red absorbing form of phytochrome after prolonged imbibition at temperatures >32°C. The enhanced sensitivity developed at 40°C could be reversed by subsequent treatment at 20°C and similarly reestablished by repeating a 40°C treatment. It is concluded that relative sensitivity to the far-red absorbing form of phytochrome may be readily manipulated in seeds of A. albus.

Evidence has accumulated that seed germination of several species of Amaranthus is promoted by single or repeated short exposures to white light or R1 and inhibited by continuous white light or exposures to FR irradiation (8–10). The photoregulation of germination has been attributed to P (10, 19). Also, seeds of Amaranthus germinate best at high temperatures (1, 2, 5, 13, 16, 19). The relation between temperature and P control of germination has been studied in Amaranthus retroflexus L. seeds where changes in light sensitivity have been attributed to changes in levels of total P (20). It has also been suggested that Pfr and temperature interact at a common point, a melanin, as an early event in the processes leading to germination (6, 21, 22). This paper reports experiments on the interaction of light and high temperature treatments on Amaranthus albus seeds which suggest that increased sensitivity to Pfr results from a change in the proposed Pfr interactant.

MATERIALS AND METHODS

Mature Amaranthus albus seeds were collected near Beltsville, MD, in 1973 and stored at −20°C in sealed polyethylene containers until use. Lots of 100 seeds were imbibed in 9-cm Petri dishes with two Whatman No. 3 filter papers moistened with 7.5 ml of distilled H2O. Seeds were held in darkness in black cloth bags at the indicated temperature ±1°C in germination cabinets. Suppression of dark germination was obtained by placing seeds under CIL for 24 h. R irradiations were from cool-white fluorescent tubes filtered to give broad band R radiation of 246 µW cm−2 in the 600- to 700-nm range at seed level. FR irradiations were from filtered incandescent sources giving 360 µW cm−2 in the 700- to 840-nm range. Germination at 30°C was assessed 3 d after irradiation. All treatments were run in duplicate and each experiment was repeated at least once. The results were presented as mean values ± SD.

RESULTS

Seeds of A. albus were germinated at temperatures from 20° to 40°C in darkness. Germination was between 50 and 60% at 25, 30, or 35°C constant at 20/30°C alternating but fell to 30% at 20 and 40°C constant. After periods of imbibition in darkness at 25, 30, 35, or 40°C, seeds were irradiated with 5-min R and allowed to germinate at 30°C (Fig. 1). The R irradiation markedly increased germination only when dark imbibition temperatures were 35°C or higher. Dark imbibition at 20 or 40°C decreased subsequent germination at 30°C if no R was given.

To discern clearly the influence of temperature on changes in photosensitivity of A. albus seeds, it was necessary to reduce dark germination. A pretreatment in CIL was examined since this is known to reduce germination of Amaranthus seeds (8, 10). In addition, since incandescent light established a relatively high Pfr/P ratio (17) that might later influence germination, a brief FR irradiation terminating the CIL pretreatment was tested (Fig. 2). Inhibition of A. albus germination by CIL depended on the duration of the CIL and the temperature during exposure. Generally, longer periods of CIL were necessary to reduce germination at 20°C than at 25°C. Dark germination was adequately suppressed by a terminal FR irradiation in shorter CIL pretreatments, regardless of the temperature. In further experiments, the pretreatment of 24-h CIL at 25°C followed by 5-min FR (0 time) was adopted as standard procedure. A brief (5 min) R irradiation immediately following the CIL and FR did not increase germination (data not shown) establishing that pretreated seeds were of low photosensitivity.

Use of the CIL pretreatment enabled further examination of the changes in light sensitivity affected by temperature. Pretreated seeds were held at several temperatures for various durations after the CIL plus FR treatment and then irradiated with R (Fig. 3). Except for sharply reduced dark germination, the data agree with those presented in Figure 1, namely, that dark treatment at temperatures >30°C is required to elicit maximum responsiveness to R irradiation. Strikingly, R irradiation increased germination of seeds held above 32°C but not below, the promotion being enhanced with increased duration of dark treatment and increasing temperature to 40°C. Dark germination was very low and did not vary in response to temperature treatment. Sensitization to R, therefore, is initiated by high temperatures. The high responsiveness to R prompted the question of whether high temperature treatment would also induce responsiveness to brief FR irradiation, which establishes a low (2–3%) level of Pfr at photoequilibrium (17). Results (Table 1) show that FR-treated seeds behave similarly to R-treated seeds in that response to FR
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FIG. 1. Percentage of germination of A. albus seeds following dark imbibition at several temperatures for various periods when irradiated or not with R. Seeds were imbibed in darkness for indicated hours at different temperatures, irradiated (■) or not (□) with 5-min R, and germinated at 30°C in dark for 3 d.

also is related to dark treatment temperature. FR irradiation did not influence the germination of seeds held below 35°C. Germination promotion by FR also required long periods of exposure to high temperatures for maximum response of A. albus seeds. Involvement of phytochrome in the photoresponses of A. albus seeds, as classically shown by R-FR reversals, may be shown in seeds that require more than the 2 to 3% Pfr formed by brief FR for germination. For example, pretreated A. albus seeds held at 40°C for 24 h germinated <1% in darkness, 51% after R, 8% after FR, and 11% after a R-FR irradiation.

To discern the relationships between [Pfr] and [P] where high temperature effects were involved, pretreated A. albus seeds were exposed to continuous FR irradiation at 40°C (Table II). Such treatment might be expected to reduce [P] so that the possibility of P synthesis in darkness as a cause for increased responsiveness to irradiation could be tested (3, 20). The data show that continuous FR during high-temperature treatment prevented development of responsiveness to subsequent brief FR irradiation compared to that of dark-held seeds. A saturating R irradiation (5 min) given immediately after seeds were exposed to the continuous FR treatment also failed to increase the A. albus germination (results not shown).

A loss in responsiveness to R irradiation was found when seeds

FIG. 2. Effect of duration of pretreatment at 20 or 25°C in CIL on percentage of germination of A. albus seeds. Seeds were imbibed at 20 or 25°C in CIL for indicated duration, irradiated or not with 5-min FR, and germinated at 30°C in dark for 3 d.

FIG. 3. Effect of duration of various temperatures on percentage of germination of A. albus seeds after R irradiation. Seeds were held at different temperatures for the indicated times following the 24-h CIL pretreatment and terminal 5-min FR irradiation. At the end of the indicated period, seeds were irradiated (■) or not (□) with 5-min R and germinated at 30°C for 3 d.

Table 1. Effect of Duration of Various Temperatures on Percentage of Germination of A. albus Seeds after Brief FR Irradiation

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>2 ± 1</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>48</td>
<td>2 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
<td>58 ± 10</td>
</tr>
<tr>
<td>96</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>45 ± 6</td>
<td>80 ± 9</td>
</tr>
</tbody>
</table>
Table II. Effect of Duration of Continuous FR Irradiation or Darkness on Percentage of Germination Response of A. albus Seeds after FR Irradiation

Seeds were held at 40°C in darkness or continuous FR irradiation for the indicated duration after the 24-h CIL and 5-min FR pretreatment. At the end of these periods, seeds were irradiated with 5-min FR and germinated at 30°C for 3 d.

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>Germination Response</th>
<th>Darkness (%)</th>
<th>Continuous FR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>12 ± 7</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>54 ± 6</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>78 ± 5</td>
<td>4 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 4. Effect of reduced temperatures following a high-temperature treatment on percentage of germination of A. albus seeds after FR irradiation. Seeds were held at 40°C for 2 d after the 24-h CIL pretreatment and 5-min FR irradiation, then transferred to indicated temperatures for different times. A 5-min R irradiation was then given and the seeds germinated at 30°C for 3 d.

Table III. Effect of 40°C Dark Treatment on Reversal of the Depressing Effect of Either 40°C in Continuous FR for 48 Hours or 20°C in Darkness for 48 Hours on Percentage of Germination of A. Albus Seeds after R Irradiation

Seeds were held at 40°C for 2 d after the 24-h CIL pretreatment and 5-min FR irradiation, then transferred to either darkness at 20°C or continuous FR for 48 h. Subsequently, seeds were held at 40°C in darkness for 0, 24, 48, or 96 h, given 5-min R, and germinated at 30°C for 3 d.

<table>
<thead>
<tr>
<th>Duration at 40°C (h)</th>
<th>Germination Response</th>
<th>Darkness (%)</th>
<th>Continuous FR (40°C) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>81 ± 3</td>
<td>64 ± 5</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>87 ± 10</td>
<td>94 ± 6</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>92 ± 3</td>
<td>94 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

Further studies have shown that continuous FR treatment to yield high responsiveness were then shifted to lower temperatures (Fig. 4). The decay of the promotive effect from a 2-d 40°C treatment began immediately after seeds were transferred to temperatures of 35°C or lower. Response to RFR irrations declined more rapidly at 30°C and reached a low level after 16 h, whereas at 35°C, responsiveness decayed gradually for 8 h and then increased to the original level. However, a complete reversal of the depressing effect of a 48-h 20°C darkness treatment could be obtained by holding the 20°C-treated seeds at 40°C in darkness (Table III). Similarly, after 48-h continuous FR treatment, responsiveness could be restored to its original level by subjecting seeds to 40°C in darkness for 48 h or more (Table III).

**Discussion**

As with other species of *Amaranthus* previously investigated (9, 10, 20), germination of *A. albus* seeds displayed sensitivity to both temperature and light. In *A. albus* seeds, high imbibition temperatures enhanced responsiveness to light-stimulated germination (Fig. 3; Table I). The enhanced P stimulation required temperatures of >32°C and dark treatment for >24 h (Fig. 3; Table I). Similar results have been obtained by Taylorson and Hendricks (20) in *Amaranthus retroflexus* seeds. Another major point was that an apparent reversal of the enhanced sensitivity to light occurred rapidly when enhanced seeds were transferred to lower temperatures (Fig. 4). Such a reversal has not been heretofore demonstrated.

Other points shown are that exposure to continuous FR during high-temperature treatment prevents development of enhanced sensitivity (Table II) and that a second high temperature incubation causing reversal of enhanced responsiveness by 20°C, or a period in darkness at high temperature following prolonged FR, reestablishes enhanced sensitivity (Table III). Such behavior enables a high degree of control over photosensitivity.

Previous work in *A. retroflexus* (20) indicated enhanced responsiveness to R irradiation could be attributed to an accelerated synthesis of P. In seeds of *Rumex crispus* L. and *Portulaca oleracea*, Duke et al. (3) suggested instead that variation in the levels of the proposed P interactant was a more tenable explanation for change in responsiveness to R.

In *A. albus* seeds, since continuous FR prevented enhanced responsiveness (Table II) and can cause destruction of P in other tissues (11), one could speculate that increased P leads to enhanced sensitivity. However, assuming that FR reduced P, it is possible that increased interactant could only be expressed in dark-treated seeds. Thus, the question of increased P affecting sensitivity remains open.

In *A. albus*, it is notable that while establishment of an enhanced sensitivity is quite slow, reversal by temperatures <35°C is much faster (Fig. 4). Temperature-affected changes in sensitivity to irradiation are often more rapid than those occurring in *A. albus*. An abrupt upward change in temperature (temperature shift) for only a few minutes to several hours enhances responsiveness of many kinds of seeds to R irradiations, notably *Rumex* spp. (15, 18). Takaki et al. (18) and Hand et al. (4) suggest that in *Rumex obtusifolius* a temperature shift likely acts on a Pfr interactant. A similar enhancement, but instead requiring several hours of chilling, has been reported for lettuce by Vaidyanathan and Toole (23). None of these has been clearly related to a membrane effect but most suggest such an action. The implications were that temperature may alter the site at which Pfr may act. While current hypotheses on initial actions of Pfr focus on membrane involvement (12), this still has not been unequivocally shown (14).

Other evidence favoring a Pfr-interactant explanation in *A. albus* relates to the temperatures required to enhance sensitivity. According to data in Figure 3, a steep change in responsiveness...
occurs around 32°C, a temperature at which membrane-related phenomena have been shown to occur in seeds, including control of leakage of amino acids (5), transitions of isolated membrane fragments as judged by affinity of fluorescent probe molecules (7), and an apparent enhancement of Pfr action in A. retroflexus seeds based on temperature affected degree of membrane organization (6).

LITERATURE CITED
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