Phytochrome Transformation in Lettuce Seed Irradiated at Various Temperatures

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ABSTRACT

Phototransformation of phytochrome in lettuce seeds (Lactuca sativa L. var. Grand Rapids) was examined by testing germination responses of seeds irradiated at various temperatures. Temperature variations from 0 to 50 C had no influence on the germination of partially hydrated seeds (about 15% water content) irradiated with either red or far red light prior to imbibition. At −15 C far red light more effectively retarded germination than red light promoted it. No effective phototransformation was detected at −79 C or −196 C.

Low temperature treatment is known to have an inductive effect on the germination of lettuce and many other types of seeds. Photosensitive lettuce seeds having a functional phytochrome system are also affected in their germination responses following irradiation with red or far red light by preincubation at various temperatures (5, 6, 9–11). Pretreatment at low temperatures tends to mimic or enhance the promotive effects of red light, whereas high temperatures tend to repress germination. Ikuma and Thimann (6) examined this problem and interpret their data as indicating low temperature stimulation of germination through a mechanism independent of phytochrome, whereas Scheibe and Lang (10) attribute the influence of low temperature to prevention of the transformation of physiologically active phytochrome to an inactive form. Recent in vitro studies of the phototransformation of phytochrome at various temperatures (1, 7, 8) demonstrate phototransformation over a wide temperature range. Low temperature observations of Pratt and Butler (8) indicate that the physiologically active phytochrome Pfr is formed from red light-activated Pr through a series of up to five dark reaction stages. While phototransformed intermediates are detectable at −196 C, it is necessary to warm the preparations for complete dark relaxation to Pfr. Reverse transformations occur at low temperatures with far red light; however, the pathway appears to be different, with no dark intermediates in common between the two (7). Spruit (12, 13) has examined in vivo phytochrome transformations in pea plumules at low temperatures, but the cell-free results are difficult to compare with those obtained from whole tissue.

Phototransformation has been shown to occur in either direction in lettuce seeds with as little as 15% seed water, but not at all in seeds with only 7% seed water content (3, 4). Observations of the temperature relations of phytochrome transformation in relatively dry seeds could yield information about the influence of various temperature treatments on phytochrome in vivo and whether or not germination responses of seeds irradiated at various temperatures parallel observed spectral transformations. We carried out a series of germination tests in which low water content seeds were irradiated at various temperatures. Our results indicate maximum phototransformation of endogenous phytochrome over a temperature range of 0 to 50 C; some inhibition of Pr → Pfr, but not the reverse at −15 C; and no phototransformation in either direction at −79 C or below.

MATERIALS AND METHODS

Grand Rapids lettuce seeds were adjusted to about 15% water content using the methods of Hsiao and Vidauber (3). Irradiation was with light of 660 nm (red) and 724 nm (far red) peak wavelengths with 6 × 104 ergs cm−2 sec−1 for 1 min in either wavelength using interference filters (Balzers, Lichtenstein) also according to Hsiao and Vidauber (3).

Temperature variations were accomplished by immersing vials containing the seeds in either water baths (0 C and above), glycerol-Dry Ice mixtures, or liquid N2 until temperature equilibration occurred as determined to −15 C by a mercury thermometer placed in water- or brine-filled vials. At the lower temperatures equilibration times were estimated by extrapolation.

Some seeds were given an initial red or far red irradiation at 20 C on achieving about 15% water content. Others received no light at this time. The seeds were then brought to the experimental temperature (shown in Table 1) and again given light. Irradiation was with red light if far red was given initially or with far red if the first irradiation was red. Of the seeds receiving no initial irradiation some were given red, others far red, and the remainder received no light. Germination by seeds receiving these treatments is shown in lines A of Table I.

Paralleling the above treatments, some seeds were treated identically, except that light was not given while the seeds were at the experimental temperature, but was given only after the seeds were returned to 20 C and stored 12 hr in darkness. This treatment was to detect possible effects of temperature variation in the absence of light. Germination by these seeds is shown in lines B of Table I.

Immediately upon completion of a series of treatments, the seeds were transferred for dark germination tests to 5-cm Petri dishes lined with two discs of Whatman No. 1 filter paper, containing 1.5 ml of distilled water. Germinations were scored as those seeds in which the radicle had visibly penetrated the testa after 24 hr of imbibition. All experiments were carried out with three replicates of 33 seeds, and germination percentages represent the arithmetical mean of the replicates.

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Table I. Effects of Various Temperatures on Phytochrome-potentiated Grand Rapids Lettuce Seed Germination

In lines A the initial light treatment, when given, was on adjustment of seed water content to about 15% at 20°C; any final irradiation was at the experimental temperature shown. In lines B the initial irradiation was as in A; the seeds were then brought to equilibrium at the experimental temperature but not irradiated; after temperature exposure they were stored 12 hr in closed vials in darkness at 20°C; final irradiation was given at the end of the storage period. Immediately on completion of any series of treatments the seeds were imbibed at 20°C in Petri dishes for germination tests. All operations were carried out either in darkness or with a dim green safelight (Kodak No. 3).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Germination in 24 Hr ± SE</th>
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<tbody>
<tr>
<td></td>
<td>R + FR</td>
</tr>
<tr>
<td>50°C</td>
<td>A 6 ± 1</td>
</tr>
<tr>
<td></td>
<td>B 6 ± 3</td>
</tr>
<tr>
<td>35°C</td>
<td>A 7 ± 3</td>
</tr>
<tr>
<td></td>
<td>B 6 ± 1</td>
</tr>
<tr>
<td>20°C</td>
<td>A 7 ± 4</td>
</tr>
<tr>
<td></td>
<td>B 7 ± 4</td>
</tr>
<tr>
<td>0°C</td>
<td>A 9 ± 4</td>
</tr>
<tr>
<td></td>
<td>B 7 ± 3</td>
</tr>
<tr>
<td>−15°C</td>
<td>A 8 ± 2</td>
</tr>
<tr>
<td></td>
<td>B 7 ± 3</td>
</tr>
<tr>
<td>−79°C</td>
<td>A 81 ± 6</td>
</tr>
<tr>
<td></td>
<td>B 7 ± 3</td>
</tr>
<tr>
<td>−196°C</td>
<td>A 87 ± 5</td>
</tr>
<tr>
<td></td>
<td>B 8 ± 5</td>
</tr>
</tbody>
</table>

1 R: red light; FR: far red light.
2 D: darkness.

RESULTS AND DISCUSSION

Table I summarizes results of the various germination tests. Photoreversibility in either direction clearly persists over the temperature range of 0 to 50°C. Red light at −15°C is less effective in promoting germination than at the higher temperatures, but there is little effect of −15°C on far red retardation. Phototransformation in seeds irradiated at −79°C or −196°C is essentially blocked in either direction, although there may be a weak response to far red light at the higher temperature.

Hsiao and Vidaver (4) have shown previously that lettuce seeds with about 15% water content or less may be stored for at least 24 hr with no loss in effectiveness of previous irradiations. Therefore germination responses of seeds exposed to any experimental temperature used here and returned to 20°C for the 12 hr period would not be expected to be affected by the storage. Inspection of line B in Table I for each temperature does indeed show that complete photoreversibility persists regardless of any intervening temperature treatment. Comparison of germination percentages in seeds irradiated at the experimental temperature (A) with those returned to 20°C for 12 hr before irradiation (B) shows that exposures to either high or low temperatures in themselves had no detectable effect on subsequent germination.

Our data appear to support the contention of Ikuma and Thimann (6) that the mechanism by which the commonly reported effects of temperature variations on germination is independent of the phytochrome system. The data give no information regarding the fate of Pfr following its transformation at the various temperatures, and therefore neither support nor contradict the hypothesis of Scheibe and Lang (10) that low temperature treatment enhances germination by preventing thermal destruction of Pfr, nor does it preclude the possibility of phytochrome interacting with a cofactor formed during imbibition, as proposed by Roth-Berjerano et al. (9).

Between 0 and 50°C, phototransformation of phytochrome appears to be temperature-insensitive, suggestive of a physical rather than a biochemical process. At temperatures below 0°C, the process is temperature-sensitive. The explanation for this variation is uncertain.

At −15°C promotion of germination by red light appears less effective than retardation by far red. This is particularly evident when initial irradiation was with far red light. Only 72% of far red preirradiated at 20°C seeds germinated after having been given red light at −15°C compared to 81% of those not preirradiated. On the other hand, far red light was maximally effective at −15°C regardless of preillumination. This result is opposite to that reported for the influence of water content on photoreversibility by Hsiao and Vidaver (3). Phototransformation by red light occurs with less water content (approximately 10%) than it does with far red (about 15%). The inhibition of phototransformation at low temperature can thus probably not be attributed to a dehydration of the phytochrome system through freezing of seed water. Nor does over-all seed water content appear to have been decreased by low temperature treatments, since the inhibition of phototransformation is completely reversed by raising the temperature; no water need be added to the seeds.

There are points of coincidence between the physiological germination tests and spectral data. The fact that Pfr → Pr photoconversions took place at lower temperatures in seeds than Pr → Pfr may imply different pathways for the forward and reverse transformations, as has been shown for the pigment in vitro (2, 8). Spruit (12) has also shown in pea plumules that the Pfr → Pr transformation occurs at lower temperatures than the reverse.

Germination responses of these seeds at low temperature do not seem to parallel the observations of Pratt and Butler (7, 8), and Spruit (12, 13), in that physiologically functional Pfr does not appear to be formed from the dark decay on warming of photoproducts produced at temperatures as low as −196°C. Our seeds were warmed to 20°C for the 24-hr imbibition fol-
lowing low temperature treatment, which should be ample time for thermal relaxation of photoproducts to occur. Nevertheless, expected germinations did not take place with seeds irradiated at the low temperatures.

Under normal growing conditions the germination of most if not all seed species is influenced by temperature. However, the data presented here indicate that commonly reported responses to temperature variations are independent of temperature effects on the transformation of phytochrome.

LITERATURE CITED