Mediation of Phytochrome in the Inductive Action of Low Temperature on Dark Germination of Lettuce Seed at Supra-Optimal Temperature[1]

Nurit Roth-Bejerano, D. Koller, and M. Negbi

The Negev Institute for Arid Zone Research, Be'er Sheva, and Botany Department, The Hebrew University of Jerusalem, Israel

Received January 25, 1966.

Summary. The induction of dark germination in light-requiring lettuce (*Lactuca sativa*) seed at supraoptimal temperatures by cold treatment (in darkness) was partly reversed by a brief far-red irradiation made at time of transfer, and even more so when the irradiation was made at the beginning of the cold pretreatment. When the inhibitory far-red irradiation was followed by additional cold treatment, the promotion was greatly restored. The promotive effects of brief irradiations with red light were further enhanced by a following cold period, before transfer to the supraoptimal temperature. These results are interpreted as indicating that the active (far-red absorbing) form of phytochrome is pre-existing in the dry seed, and interacts with a co-factor which is built-up during imbibition. The rate of build-up of this co-factor, as well as of the dark inactivation of active phytochrome increase with temperature. The products of the interaction pass through a photo-labile thermo-stable phase, before becoming photo-stable as well.

Dark germination of photoblastic lettuce seed can take place at temperatures below a certain level and in absence of water-stress (4, 5), and even at higher temperatures, if preceded by relatively low temperatures (3). Toole (10) has suggested that dark germination in such seeds is due to pre-existence of the active, far-red absorbing form of phytochrome, P_{fr}, and that light is required at high temperature because this P_{fr} is thermally inactivated. This problem is dealt with in 2 recent papers, whose conclusions do not agree with each other. Thus, while Ikuma and Thimann (3) state that the action of low temperature is to stimulate germination at a point other than that controlled by phytochrome, Scheibe and Lang (9) ascribe it to the probable prevention or delay of transformation of physiologically active phytochrome to an inactive form. The present study was made as a contribution to this controversy.

Materials and Methods

The experiments were made with lettuce seed cv. Grand Rapids, which were stored throughout at 5° to 7° in darkness. Each test was carried out in quadruplicate, using 50 seeds per petri dish (5 cm diam), planted on a single layer of Whatman No. 1 filter paper, moistened with 3 ml double deionized water. Red (R) and far-red (FR) light were obtained by the filter systems described by Koller, Sachs and Negbi (7). The light source was a 150 w Phillips Attralux incandescent spot lamp, with a built-in reflector. Dark conditions were obtained by enclosing the dishes in light-proof tins. Germination at 30° was counted after 48 hours. In no case did any germination occur during the cold pretreatment.

Results and Discussion

Progressively longer periods of cold pretreatment (in darkness) at 0° allowed an increasingly larger percentage of the seeds to germinate after transfer to 30° in darkness. A brief FR irradiation at time of transfer reduced this percentage. The inhibitory action of FR was less pronounced after longer periods of cold pretreatment (table I). FR irradiation at the beginning of a 3-day cold pretreatment (allowing 30 min at 0° for imbibition and temperature equilibration, before irradiation) reduced the effectiveness of the 3-day pretreatment far below that of a 2-day nonirradiated pretreatment. FR irradiation after a 2-day cold pretreatment also greatly reduced its effectiveness, but when irradiation was followed by an additional 3-day cold treatment, promotion was nearly fully restored (table II). These results are not compatible with the hypothesis that cold treatment promotes germination by a separate pathway from that of phytochrome (3), unless one assumes

[1] This study was supported by PL 480 grant FG-Is-115 from the United States Department of Agriculture and was carried out in partial fulfillment of the requirements for a Ph.D. degree by Nurit Roth-Bejerano.

at the same time that the red-absorbing form of phytochrome, P_r, acts as inhibitor of germination and is counteracted by the promotive action of cold. Scheibe and Lang (9) provide evidence which suggests that P_r could not be acting as an inhibitor. An alternative possibility by which these data may be explained is that even after saturating dosages of FR, a small fraction of phytochrome is present as P_{fr} (6). By this reasoning, the second post-irradiation cold period increased germination not independently of phytochrome, but by preventing thermal inactivation of the small amount of P_{fr} formed by the FR irradiation. In that case, qualitatively the same, though quantitatively larger effects of post-irradiation cold treatment should result from promotive irradiation with R. This was tested in the experiment summarized in table III. The results show that R irradiation promoted subsequent germination at 30° to a greater extent when applied after 3 days than after 30 minutes cold pretreatment, and that this promotive action was further enhanced by a second, post-irradiation cold treatment. It was further found that R irradiation after 30 minutes at 30° was more promotive than at 1° (57 ± 7 vs. 11 ± 4 %, respectively), while the reverse was true after 3 days (2 ± 0 and 67 ± 3 % at 30° and 1°, respectively). This indicated that photoresponsiveness of the phytochrome system is initially low, and that its rate of increase is positively correlated with temperature. The subsequent reduction in responsiveness when dark incubation is prolonged to 3 days is a manifestation of high-temperature induction of the inhibition described as skoto-dormancy (1), which is not part of the present subject.

Table I. *Dark Germination of Lettuce Seed at 30°: Induction by Low-Temperature Pretreatment, as Affected by FR-Irradiation at Time of Transfer*

Duration of pretreatment*	% Dark-germination at 30° Expt 1 —FR	Expt 1 +FR**	Expt 2 —FR	Expt 2 +FR**
0 Min	3 ± 1	...	...	...
30 Min	1 ± 0	1 ± 1	1 ± 0	1 ± 1
1 Day	9 ± 3	1 ± 0	...	...
2 Days	...	...	57 ± 6	10 ± 1
3 Days	83 ± 2	31 ± 2	65 ± 7	39 ± 6
6 Days	83 ± 1	...	77 ± 6	64 ± 7

* At 0° in the dark.
** 5 Minutes at time of transfer from 0° to 30°.

Table II. *Dark Germination of Lettuce Seed at 30°: Recovery from FR Reversal of Inductive Effects of Cold Pretreatment by Additional, Post-Irradiation Cold Treatment*

Duration of initial cold-treatment*	% Dark germination at 30° after indicated additional pretreatment None	FR**	FR**→ 3 days cold treatment*
0 Min	2 ± 0	...	...
30 Min	2 ± 0	1 ± 0	34 ± 4
2 Days	84 ± 3	24 ± 6	75 ± 4

* At 0°.
** 5 Minutes at end of initial cold treatment.

Table III. *Dark Germination of Lettuce Seed at 30°: Additive Effects of R Irradiation with Preceding and Following Cold Period During Induction*

Duration of initial cold treatment*	% Dark germination at 30° after indicated additional pretreatment None	R**	R**→ 3 days cold treatment*
30 Min	2 ± 0	18 ± 8	65 ± 4
3 Days	48 ± 7	79 ± 3	86 ± 3
0 Min	3 ± 1	...	...

* At 1°.
** 5 Minutes at end of initial cold treatment.

The results in table II and III, therefore indicate that 2 processes occur simultaneously during incubation, even at near-freezing temperatures. One is concerned with an increase in responsiveness of the phytochrome system towards the promotive action of R, as well as to the small part of FR which is also promotive. This has already been suggested by results with *Oryzopsis miliacea* (8). The promotion observed by Scheibe and Lang (9) when 2 FR irradiations were separated from each other by exposures to 37° not exceeding 4 hours (their table VI) may be explained on the same basis, rather than by promotive action of the high temperature. On the contrary, exposures to 37° longer than 4 hours were probably inducing skoto-dormancy, i.e. reducing the responsiveness to the second FR irradiation. The increase in responsiveness of the phytochrome system in *Oryzopsis* was ascribed to increase in pigment concentration (8, 10). In lettuce this is undoubtedly not the case, as dark germination may reach 100 %, provided temperature is sufficiently low (2). Therefore, what causes the increase is buildup of some factor with which P_{fr} interacts, as suggested for *Rumex obtusifolius* by Vicente, Engelhardt and Silberschmidt (11).

We may now turn to the other process which occurs during incubation, even at near-freezing temperatures, and suggest that this is concerned with the interaction between P_{fr} (whether preexistent, or neogenic) with this cofactor. At least 2 phases can be distinguished in the interaction between this cofactor and P_{fr} on the basis of the present results. During the first of these the product of the interaction is thermo-stable (at 30°), but photo-labile. A this stage it can be inactivated by FR and is therefore most probably still complexed with P_{fr}. During the second phase it becomes photo-stable as well. At the start of imbibition the product of interaction either does not exist, or has not yet achieved thermostability, as total inactivation results from early transfer to 30° after FR irradiation.

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