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Studies of the Mechanism of Enhancement of Phytochrome-dependent Lettuce Seed Germination by Prechilling

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WILLIAM J. VANDERWOUDE AND VIVIAN K. TOOLE
Seed Research Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research, Science and Education Administration, United States Department of Agriculture, Beltsville, Maryland

ABSTRACT

Temperature and kinetic studies were performed to examine the mechanism by which prechilling stimulates phytochrome-dependent seed germination in lettuce, *Lactuca sativa*, L. cv. Grand Rapids. Imbibed seeds were given a short far red irradiation and one day of dark incubation at 20 C to establish very low levels of the far red-absorbing form of phytochrome—(Pfr). Germination was greatly stimulated by subsequent prechilling treatments when they were followed by a second short far red irradiation. Prechilling therefore increased germination sensitivity to the low, normally inhibitory Pfr levels established by far red irradiation. This sensitivity increased with lowered prechilling temperature to a maximum near 4 C. It was linearly dependent upon duration of prechilling at 4 C up to a near maximal response at 10 hours, and it decayed in a converse manner when seeds were returned to 20 C after 10 hours at 4 C. Prechilling also increased germination responses to subsequent periods of high levels of Pfr which were initiated by red and terminated by far red irradiations. High Pfr periods adequate to promote the germination of unchilled seeds produced sharp inflections at 18 C in the dependence of germination on prechilling temperature. Rates of phytochrome potentiation of germination were not affected by prechilling. The response to prechilling fit a mechanism involving homeoviscous adaptation of membrane lipids to temperature.

Initial incubation at low temperatures enhances the germination of seeds of many species (12, 24). Several hours or a few days of low temperature incubation, referred to as prechilling, promote the germination of nondeep dormant seeds (15). Light is often a factor in relieving dormancy of such seeds and prechilling reduces the requirement for light. The germination in darkness of positively sensitive lettuce seeds (achenes) is increased by prechilling (9, 20, 28, 29). Prolonged incubation of these seeds at constant low temperature also results in high levels of dark germination (4, 8, 21, 23). Dark germination requires the presence of physiologically active phytochrome, Pfr (14). The prechilling enhancement of dark germination has been attributed to a reduced rate of dark reversion of Pfr to physiologically inactive Pr at the low temperature (21, 25). We report temperature and kinetic studies which indicate that increased sensitivities to Pfr resulting from altered membrane properties underlie the enhancement of phytochrome-dependent seed germination by prechilling.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* L.) seeds, Tip Burn Resistant strain of cv. Grand Rapids, lot number 12710–18639, (Ferry Morse Seed Co., Mountain View, Calif.[1]) were obtained in June 1977 and stored in sealed containers at −18 C. Germination of these seeds at 20 C was 40% in darkness and 99% after brief R[2] irradiation.

Light Sources. A green safelight was used only during placement of seeds on thermogradient plates. It consisted of one General Electric F40G (green) fluorescent lamp in a plastic safety shield wrapped with two layers of Roscolene No. 877 (medium blue-green) and one of No. 874 (medium green) (Rosco Laboratories, Inc., Port Chester, N.Y.). At seed level, 140 cm from the source, total irradiance was 3.3 $\mu w/cm^2$, 95% of which was between 500 and 540 nm.

The R and FR sources consisted, respectively, of 12 red and 12 black light IR, 122 cm 1.5 amp, special phosphor fluorescent lamps (Controlled Environment Systems, Inc., Rockville, Md.). The two lamps were positioned alternately in a single housing which could be moved on overhead tracks to permit irradiation of any of three thermogradient plates. Lamps were placed in plastic safety shields which absorbed UV irradiation and prevented excitation of phosphors in red lamps when the FR source was used. Light was transmitted through two layers of red cellophane and a Plexiglas diffuser located one meter above seed level.

The emission spectra of the light sources (Fig. 1) were measured with a cosine-corrected spectroradiometer developed by K. H. Norris and the Instrumentation Research Laboratory at Beltsville. Total irradiances at seed level were 450 $\mu w/cm^2$ (600–700 nm) for the R source and 190 $\mu w/cm^2$ (700–800 nm) for the FR source. After incubation of seeds at 20 C for 24 h, germination at 20 C was fully promoted by 15 s of R irradiation. Four min of FR irradiation was required under these same conditions to reverse fully the promotion by 5 min of R light.

Thermogradient Studies. The simultaneous study of germination responses to many temperatures was simplified by the use of thermogradient plates (10, 11). The 76- × 76-cm plate surface was covered with a water-saturated substratum consisting of one thickness of Kimpac tissue between two thicknesses of germination blotting paper. Thirty parallel lines, spaced 2.54 cm apart, were drawn perpendicular to the temperature gradient on an upper layer of Whatman No. 3MM filter paper. Temperatures were maintained within 0.5 C of indicated values and were monitored with 12 or 24 thermocouples placed in the upper surface of the substratum and attached to multichannel chart recorders. The clear Plexiglas top of the plate remained in place during irradiations and was covered with several layers of black cloth when light was to be excluded.

Seeds were placed on plates at 20 C under green light, 150 seeds

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[2] Abbreviations: R: red; FR: far red.

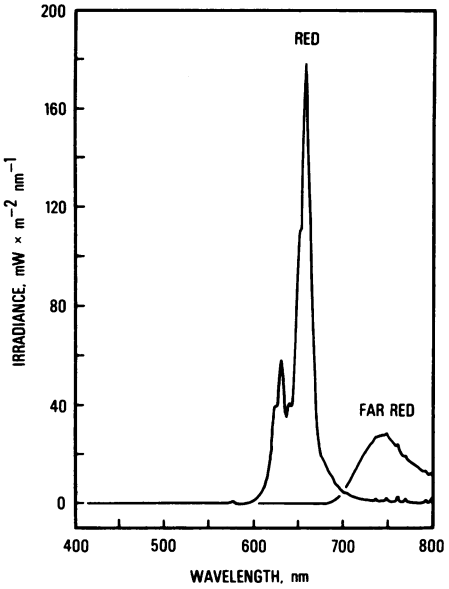

FIG. 1. Emission spectra of the R and FR light sources. Measurements were made at seed level, 1 m from the sources, with six lamps on.

being evenly distributed along each of the 30 lines. Five-min FR irradiations were given at the middle and end of the 1-h planting period and also 1 h after planting was complete. The seeds then remained in darkness at 20 C until 24 h after planting before receiving further treatment. Plate temperatures where shifted from 20 C to a thermal gradient and returned to 20 C by adjustment of the temperature regulators for the circulating water baths attached to the plates. (The temperatures which were given between preincubation and postincubation at 20 C are referred to as "treatment" temperatures.) Auxiliary heating and cooling devices hastened the temperature shifts. The shift to an equilibrated temperature gradient was performed in 2 h, whereas the return to 20 C required 1.5 h. Seeds were incubated at 20 C in darkness for 48 h after the last light treatment. Germination was then scored on the basis of visible radicle emergence. Experiments were performed twice and data are presented from single experiments as the percent germination of the 150 seeds at each treatment temperature.

Kinetic Studies. One hundred seeds were evenly distributed on a circle of dry, cotton polyester cloth cemented to a 9-cm-diameter plastic hoop (cut from a disposable Petri dish). Imbibition was begun in darkness by transferring these dishes to covered plastic sandwich boxes containing one thickness each of Kimpac and germination blotting paper and 90 ml water at 20 C. A 5-min FR irradiation was given after 1 h. After 23 h, rapid equilibration of seeds at 4 C was accomplished by transferring the cloth dishes in darkness to boxes equilibrated at this temperature. Similarly, to terminate the prechilling period the cloth dishes were returned to boxes at 20 C. Boxes were placed in black cloth bags when necessary to exclude light. Four replicates of each treatment were examined.

RESULTS AND DISCUSSION

Effect of Temperature on Phytochrome Potentiation of Germination. This study of prechilling arose out of our initial efforts to study the processes which underlie phytochrome potentiation of germination by examining the temperature dependence of the rate of potentiation. We attempted to restrict the influence of temperature to just these processes by exposing seeds to treatment temperatures only during defined periods of high Pfr levels (initiated by R and terminated by FR irradiations).

Figures 2 and 3 and their legends present the results and details

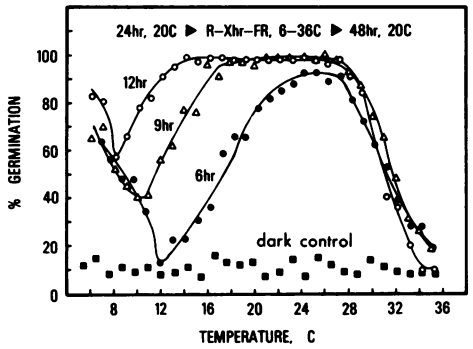

FIG. 2. Effect of temperature on phytochrome potentiation of germination. All experiments, unless otherwise indicated, employed FR irradiations during imbibition and dark preincubations at 20 C for 24 h to reduce levels of Pfr and dark germination. The temperature gradient was then established, and a 5-min R irradiation given. After 6, 9, or 12 h seeds were irradiated 8 min with FR and returned to 20 C. Germination was scored in all experiments 48 h after the last irradiation. Dark control seeds received a 6-h exposure to the treatment temperatures and were scored for germination 48 h after their return to 20 C.

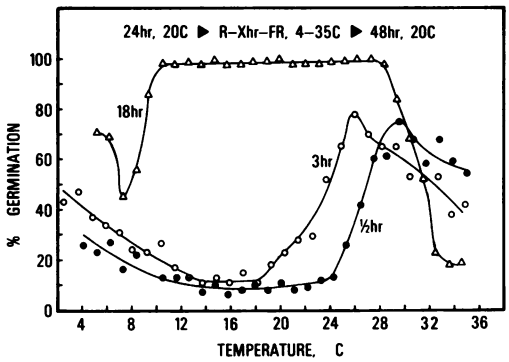

FIG. 3. Effect of temperature on phytochrome potentiation of germination. Experiments were performed as those of Figure 2 but with 0.5, 3, or 18 h between R and FR irradiations.

of several such experiments. The pretreatment with FR irradiation effectively lowered dark germination to an average level of 11%. A 6-h exposure to the treatment temperatures had no apparent influence on dark germination (Fig. 2). It also did not alter the maximum germination (99%) promoted by R irradiation given at the beginning of the treatment-temperature period (data not shown). The responses to treatment temperature given during the various periods of high Pfr were complex, particularly below 14 C and above 24 C. At the intermediate temperatures a Q_{10} of 3.1 was estimated for the rate of potentiation from the temperatures at half-maximal promotion of germination above dark controls (55%) by 6- and 30-h high Pfr periods (17.9 and 24.3 C, respectively). The complex interactions which were apparent at lower and higher temperatures could also have influenced germination responses at the intermediate temperatures. A more detailed thermal analysis of phytochrome potentiation of germination was therefore deferred pending an understanding of these interactions.

Temperatures below 14 C resulted in germination responses to 0.5 and 3 h of high Pfr which were above the dark germination level responses found at 14–18 C (Fig. 3). High Pfr periods of 6 h or more produced responses which were closely coincident at 6 to 10 C (Fig. 2). These findings are explicable if the enhanced germination of seeds exposed to low temperatures was not due to phytochrome potentiation during the high Pfr periods, but rather to subsequent potentiation of germination at 20 C by the low level of Pfr produced by the FR irradiation which terminated these

periods. Low temperature treatments apparently enhanced subsequent sensitivity of the germination response at 20 C to low Pfr levels. Up to 6 h, increasing periods of high Pfr (and exposure at the treatment temperatures) produced similar responses at 12 C but increased responses at lower temperatures. These results could be explained if the enhancement of sensitivity to low Pfr levels depended on the duration of exposure to low temperature.

Promotion of Germination by FR Irradiation after Prechilling. We tested the foregoing explanations of the responses to low temperature treatments by giving seeds a short FR irradiation at 20 C after 6 h at treatment temperatures of 1–21 C (Fig. 4). Irradiation with FR promoted germination of seeds subjected to treatment temperatures below 13 C but not above. The promotion increased with decreasing temperature, reaching a maximum near 4 C. Prechilling, therefore, greatly enhanced sensitivity to the low levels of Pfr which resulted from FR irradiation. This conclusion is supported by dose-response studies in which sensitivity to narrow band 660 nm R irradiation was enhanced 2,000-fold by 10 h of prechilling at 4 C (VanDerWoude, unpublished). Dark germination was low and varied little in response to the treatment temperatures. The nonresponsiveness of dark controls to prechilling was attributed to dark reversion of the low levels of Pfr produced by the FR preirradiations to Pr during the 24-h dark preincubation period. Since such low levels of Pfr existed during the prechilling period, a mechanism for prechilling enhancement of germination other than that of a reduced rate of dark reversion of Pfr to Pr at low temperatures (21, 25) was indicated.

Kinetics of Prechilling Enhancement of FR-Promoted Germination. Germination in response to FR irradiation was dependent upon the length of the prechilling period (Fig. 5). The response to FR was enhanced by as little as 1 h of prechilling at 4 C. It displayed no lag period and increased linearly up to a near maximum in seeds prechilled for 10 h. The promotive effects of prechilling at 4 C for 10 h remained during subsequent incubation at 20 C but gradually decayed (Fig. 6). The decay began immediately after transfer of the seeds to 20 C and was linear for the first 10 h. The response to FR ultimately declined to a level near that of the dark control.

Effect of Prechilling on Germination Response to Periods of High Pfr. One possible explanation for the prechilling enhancement of sensitivity to low Pfr levels was an increased rate of potentiation. We reasoned that if the rate of potentiation was high enough, seeds would attain germination potentials beyond threshold levels before the occurrence of appreciable dark reversion of Pfr after FR irradiation. We therefore examined the influence of 6 h of prechilling at 1–21 C on potentiation of germination at 20 C by various periods of high Pfr.

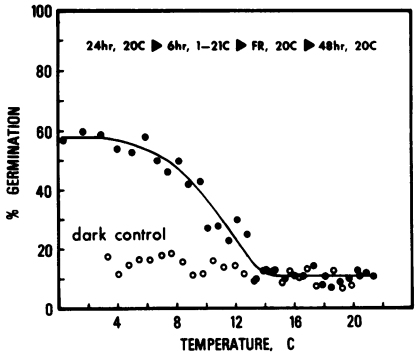

FIG. 4. Effect of various prechilling temperatures on germination responses to FR irradiation. A stable temperature gradient was established and maintained for 6 h, after the usual FR pretreatment and 24 h dark preincubation at 20 C. The temperatures were then returned to 20 C, and the seeds given an 8-min FR irradiation. Dark control seeds did not receive this final exposure to FR.

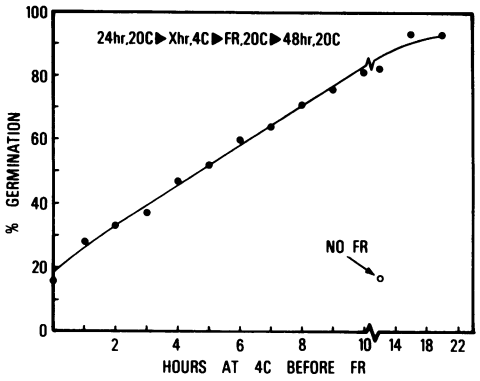

FIG. 5. Effect of duration of prechilling at 4 C on promotion of germination by FR irradiation. Seeds were incubated at 4 C for the indicated times after the usual FR pretreatment and 24-h dark incubation at 20 C. They were then returned to 20 C and irradiated 8 min with FR light.

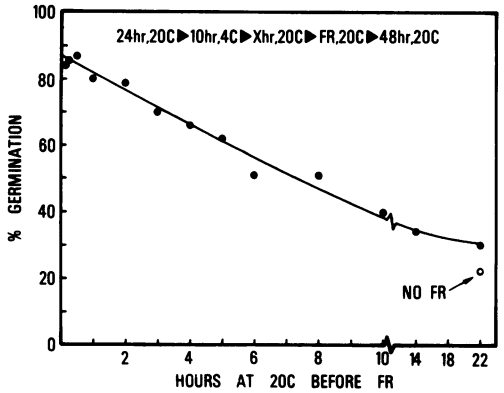

FIG. 6. Decay at 20 C of prechilling-enhanced sensitivity to FR irradiation. Seeds were prechilled for 10 h at 4 C in the manner of the experiment of Figure 5. They were then incubated at 20 C for the times indicated before being given an 8-min FR irradiation.

Germination in response to all observed periods of high Pfr was greatly enhanced by prechilling (Fig. 7). Short periods (1.5 and 3 h) of high Pfr potentiated responses which were maximal in seeds prechilled at temperatures near 4 C and which declined to dark control levels near 15 C. The minimum responses potentiated by 4.5 and 6 h of high Pfr were greater than dark control levels and allowed the physiological effects of all prechilling temperatures to be visualized as germination responses. These longer periods resulted in sharp inflections at 18 C in the relationship between germination and prechilling temperature.

Although prechilling increased germination responses to periods of high Pfr, it did not substantially alter that rate of potentiation of germination by phytochrome. This is shown in Figure 8 where the probits of per cent germination after prechilling at several temperatures are plotted *versus* the duration of the high Pfr period. In this probit analysis we assume that seeds are normally distributed relative to the potentiation period required for escape from phytochrome control of germination as indicated by the data of previous studies (2). Rates of potentiation are then given by the slopes of probit increases that begin above FR control levels. (Germination at FR control levels resulted from germination before the high Pfr period and that potentiated afterward in prechilled seeds by the terminal FR irradiation.) The rate of potentiation in unchilled seeds, obtainable only for the period of high Pfr between 4.5 and 6 h, was similar to those rates produced in prechilled seeds by periods of high Pfr between 3 and 6 h.

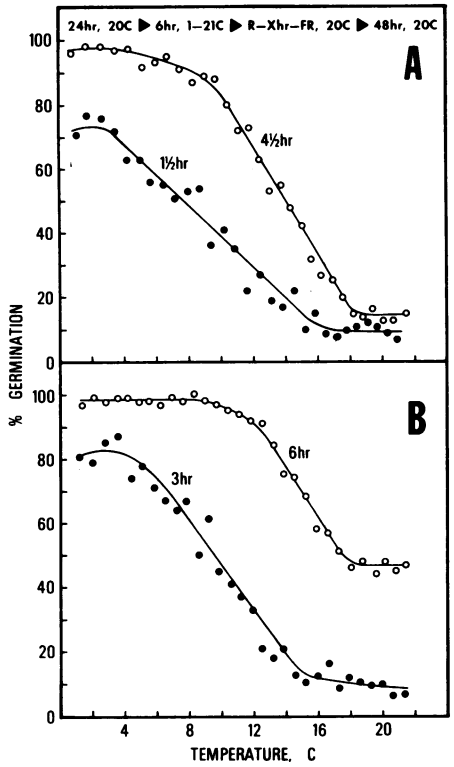

FIG. 7. Effect of various prechilling temperatures on germination responses to periods of high Pfr. Seeds received the usual FR pretreatment and 24-h preincubation at 20 C. They were then exposed to the indicated temperatures for 6 h, returned to 20 C, and given a 5-min R irradiation. An 8-min FR irradiation was then given after (A) 1.5 or 4.5 h, and (B) 3 or 6 h.

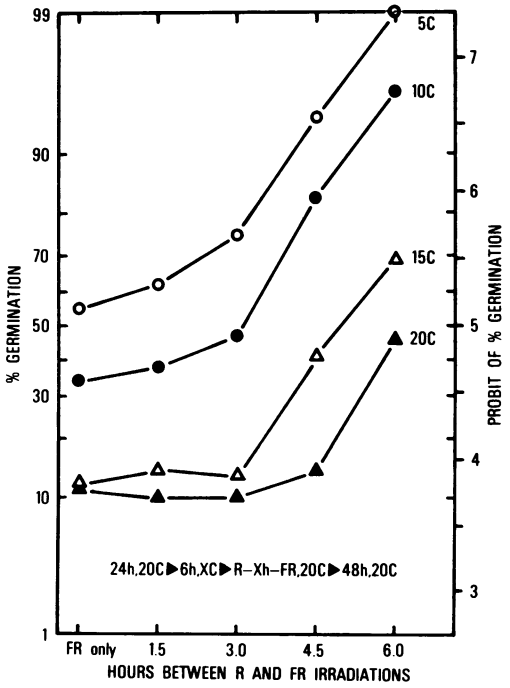

FIG. 8. Probit analysis of the effect of several prechilling temperatures on subsequent rates of phytochrome potentiation of germination. The data are drawn from three-point averaged curves of the data in Figures 4 and 7.

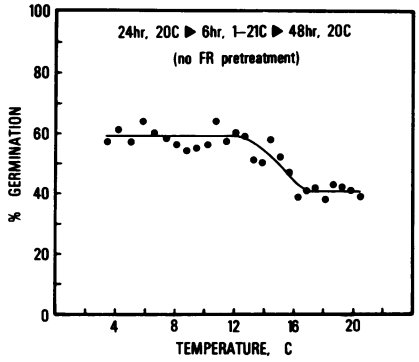

FIG. 9. Dependence of dark germination on prechilling temperature. Seeds were arranged in rows on a dry, cotton polyester cloth supported by a 70- × 70-cm wood frame. This device permitted seeds to be transferred from one thermogradient plate to another and to remain in darkness from before imbibition until scoring of germination. No FR irradiations were given. After incubation at 20 C for 24 h seeds were held at the indicated temperatures for 6 h and then returned to 20 C.

Prechilling, therefore, did not alter rates of escape from phytochrome control of germination.

As shown above (Fig. 4), germination in response to FR irradiation alone was inversely related to prechilling temperature (Fig. 8). In a similar manner, lower prechilling temperatures increasingly shortened the periods of high Pfr required to produce germination responses above FR levels. Germination was increased by high Pfr periods as short as 1.5 h. This behavior could be attributed to the addition of subthreshold levels of germination potential produced by short periods of high Pfr to those produced in prechilled seeds by the Fr irradiations which terminated the high Pfr periods. The responses of prechilled seeds to about the first 4.5 h of high Pfr may, therefore, parallel the development of subthreshold germination potentials in unchilled seeds during this period.

The above findings indicate that prechilling enhances the sensitivity to low levels of Pfr but does not alter the rate of potentiation of germination. This suggests that prechilling modifies the control of potentiation by phytochrome rather than the processes of potentiation. This contrasts with an apparent modification of the processes of potentiation by prolonged dark incubation (3).

Effect of Prechilling on Dark Germination. In the foregoing studies, where very low Pfr levels were established by pretreatment with FR irradiation and a 24-h dark preincubation, dark germination was low and little influenced by prechilling (Fig. 4). When no FR preirradiation was given (Fig. 9) the dark germination of unchilled seeds was about 40%. Germination was increased to about 60% by prechilling at 4–12 C for 6 h. These responses indicate that just as prechilling enhances the sensitivity to Pfr established by subsequent FR or R irradiation, it also enhances the sensitivity to Pfr that originates from the dry seeds. The inflection at 16 C in the temperature dependency of germination was not necessarily physiologically significant since higher prechilling temperatures may have produced increased but subthreshold germination potentials not visualized as enhanced germination responses.

DISCUSSION

Temperature-dependent transitions in the order of membrane lipids may be a primary mechanism of germination control by temperature as suggested by Hendricks and Taylorson (6, 7). They have shown close correlations of inflections in the temperature dependencies of germination, membrane probe fluorescence, and solute leakage at 28–32 C. Our findings indicate that prechilling temperatures below 18 C greatly enhance germination sensitivity

to low levels of Pfr and suggest that a membrane transition near this temperature may be involved in these responses. Biophysical evidence for such a transition is provided by the fluorescence studies of Hendricks and Taylorson (7). They found a heterogeneous membrane fraction from Grand Rapids lettuce to display an inflection near 17 C in the temperature dependency of membrane probe fluorescence. In striking analogy, the enhancement of speed of germination of dormant barley by continuous low temperature displays a sharp threshold near 17 C (19).

Membrane transitions underlie one mechanism of chilling injury in plant tissues elucidated by Lyons and Raison and their colleagues (13, 17). In this mechanism, oxidative respiratory activity is decreased relative to glycolysis at temperatures below that of a transition in mitochondrial membranes. The resulting metabolic imbalance is inversely related to temperature and leads to the accumulation of fermentation products at low temperatures. Such a mechanism is consistent with the properties of the prechilling phenomena we have reported. Its function in prechilling was suggested by the finding that ethanol promotes the germination of light-sensitive seeds (16, 26). However, prechilling caused little or no change in the low levels of ethanol, acetaldehyde, and acetone present in imbibed lettuce seeds (VanDerWoude and Taylorson, unpublished) and the function of the above mechanism in prechilling is, therefore, not indicated.

In many organisms, modifications of the saturation and chain lengths of fatty acids in phospholipids permit a return of membrane lipids to initial viscosities after a shift in temperature (1, 5, 18, 22, 27). The ubiquitous occurrence of such "homeoviscous adaption" (22) suggests that this mechanism also functions in imbibed, dormant seeds to cause the following in response to prechilling: (*a*) increased proportions of unsaturated and short chain fatty acids in membrane phospholipids related directly to the duration of prechilling and inversely to the prechilling temperature; (*b*) lower than initial membrane lipid viscosities immediately after a return to the initial temperature, the decreases in viscosity also being related directly to the duration of prechilling and inversely to the prechilling temperature; and (*c*) a return of viscosities to initial levels during subsequent incubation at the initial temperature.

The enhancement by prechilling of germination responses to FR irradiation (Figs. 4–6) paralleled these expected decreases in membrane lipid viscosities. Although biochemical and biophysical data are yet required, our results therefore suggest that a change in membrane order and lipid composition, reflected as a decrease in viscosity, is responsible for the prechilling phenomenon. This hypothesis may also extend to the promotion of light-sensitive seed germination by alternating and brief, high temperature treatments.

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