

On the Mechanism of Skotodormancy Induction in Grand Rapids Lettuce (*Lactuca sativa* L.) Seeds

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Summary

Prolonged imbibition of lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds in darkness results in a gradual loss of responsiveness to a short Red irradiation. This induction of skotodormancy was clearly shown to be temperature dependent above a critical temperature between 15 and 17.5 °C. At lower temperatures (15 and 10 °C) skotodormancy was not imposed at all, at least during the 20-day incubation period tested.

When lettuce seeds were kept under continuous Far Red, instead of being pretreated in darkness, the induction of dormancy did not attain the final level of the dark-incubated seeds. Moreover, the responsiveness to R was eventually resumed after several days in cFR and the higher the fluence rate the earlier the recovery of responsiveness. In fact, at the highest FR fluence rate used, no additional dormancy was imposed. However, in comparison to the untreated seeds, a marked delay of germination was observed for the FR pretreated ones.

Both the induction of skotodormancy and the reverse effect produced by high fluence cFR are discussed in terms of phytochrome and its postulated reaction partner X.

Key words: *Lactuca sativa* L., lettuce, photodormancy, phytochrome, seed germination, skotodormancy.

Abbreviations: cFR = continuous Far Red; FR = Far Red; R = Red.

Introduction

In a number of light-requiring seeds the responsiveness to a saturating red light pulse after reaching a maximum is progressively lost. This decline is regarded as a type of secondary dormancy called «skotodormancy». Since these seeds were already in a state of primary dormancy and they have not lost entirely their responsiveness to Red light (photosensitivity) (Georghiou and Thanos 1983), this physiological state must be considered as a deeper form of primary dormancy, rather than as a distinct type of a secondary one. Nevertheless, for reasons of convenience, «skotodormancy» will be used in this paper as well, though it would be most appropriate to apply the term «loss of responsiveness (sensitivity) to a R pulse».

The rate of decline to red responsiveness is markedly affected by temperature as reported for *Rumex* spp. (Le Deunff 1971, Taylorson and Hendricks 1973, Totterdell and Roberts

1979), *Chenopodium album* (Karssen 1970), *Sinapis arvensis* (Frankland 1976), *Arabidopsis thaliana* (Cone and Spruit 1983), and *Kalanchoe brossfeldiana* (Rethy et al. 1983). In all these cases both the extent and the rate of skotodormancy induction increase with increasing temperature.

Lettuce seed has been a preferred material of many studies related to phytochrome-mediated germination but, in contrast to the above mentioned species, skotodormancy induction was observed and studied only in a narrow range of temperatures around 25 °C (e.g. Bewley 1980). In a single case, the photosensitivity of lettuce seeds as a function of time was investigated at different temperatures (Ikuma and Thimann 1964). However, only the first phase of ascending responsiveness after the onset of imbibition was studied in that work.

The absence of data concerning lettuce seed skotodormancy induction at a wide range of temperatures (especially at lower ones) is not surprising since this type of dormancy can only occur at those temperatures at which

there is a light requirement. According to the seed germination pattern of lettuce, most Grand Rapids batches do germinate in darkness at low temperatures. However, the wide range of temperatures at which the different batches become light-requiring does not exclude the existence of batches with low dark germination at low temperatures. Such a batch was actually available in our lettuce-seed stock. Both the lack of information on the temperature dependence of skotodormancy induction in lettuce and the proposal that the onset of this type of dormancy could result from a temperature dependent loss of the P_{fr} reaction partner X or a decrease in effectiveness of the complex $P_{fr}X$ (Cone and Spruit 1983) led us to a detailed study of skotodormancy induction kinetics as a function of temperature.

The application of a prolonged FR irradiation on dark germinating lettuce seeds results in photoinhibition of germination and furthermore in a kind of secondary dormancy (photodormancy). The inhibitory effect of prolonged FR light is fluence rate dependent (Górski and Górka 1979) whereas the extent of dormancy depends upon the length of the FR duration before the eventual transfer to darkness (Boisard 1969). Prolonged FR irradiation for inhibition of germination has also been applied in primarily dormant lettuce seeds escaped from reversibility to a short FR irradiation (Mohr and Appuhn 1963, Rollin 1963, Hartmann 1966). Thus, we considered as interesting i. to study the action of prolonged FR irradiation (applied from the beginning of imbibition) on the photosensitivity changes of primarily dormant lettuce seeds and ii. to compare these changes with those observed in dark imbibed ones, towards the understanding of the mechanism of skotodormancy induction and the possible role of phytochrome in this process.

Materials and Methods

Light-requiring lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds (strictly achenes) were purchased in 1983 from Ferry Morse Seed Co., USA and were immediately stored at -20°C , in darkness, until needed for experimentation. Germination tests were carried out with 5 samples, of 50 seeds each, in Petri dishes (\varnothing 7 cm) lined with two layers of filter paper and wetted with 3 cm^3 deionised water. Each experiment was replicated at least once, thus germination data are means of at least 10 samples, from which standard errors (SE) are indicated in the table and in the figures. The criterion of germination was radicle protrusion and the germination tests ended when no additional seeds germinated.

Seeds were imbibed for various time periods either a) in darkness at constant temperatures in the range $10\text{--}30^{\circ}\text{C}$ or b) at 25°C , under cFR light of different fluence rates. Immediately after these pretreatments, seeds were given a short (10 min) R illumination (fluence rate $3\text{ W}\cdot\text{m}^{-2}$) and were subsequently transferred to darkness and left to germinate at the corresponding pretreatment temperature. Control seeds were exposed to R light 1 h after onset of imbibition and this was taken as the zero point in Figs. 1 and 3. For the calculation of half induction time (Fig. 2), the skotodormancy curves of Fig. 1 were used whereas half maximal germination was estimated as the mean of final levels of dark (Table 1) and R-induced germination (shown in Fig. 1).

The temperatures used were obtained using incubators (W. C. Heraeus GmbH, W. Germany, model BK 5060 EL) set at 10, 15, 17.5, 20, 22, 24, 28 and 30°C , maintained to within $\pm 0.1^{\circ}\text{C}$. Ex-

Table 1: Final dark germination of Grand Rapids lettuce seeds at various temperatures.

Temperature ($^{\circ}\text{C}$)	Germination (%) \pm SE
10	19.2 \pm 2.7
15	28.8 \pm 1.6
17.5	26.1 \pm 1.4
20	28.3 \pm 1.5
22	19.3 \pm 1.0
24	14.3 \pm 1.2
25	11.8 \pm 1.0
26	7.7 \pm 1.0
28	4.6 \pm 0.7
30	0.4 \pm 0.4

periments with cFR (Fig. 3) as well as time-courses of germination (Fig. 4) were carried out in plant growth cabinets (Convion, Canada) set at 25°C , maintained to within $\pm 0.5^{\circ}\text{C}$. Far red in these cabinets was obtained by filtering the light of Philips bulbs (40 W) through one red (501) and two blue (627) plexiglass filters, each 3 mm thick (Röhme GmbH, W. Germany). Different fluence rates were obtained from 1 ($0.2\text{ W}\cdot\text{m}^{-2}$), 2 ($0.4\text{ W}\cdot\text{m}^{-2}$), 4 ($0.8\text{ W}\cdot\text{m}^{-2}$), and 8 ($1.6\text{ W}\cdot\text{m}^{-2}$) bulbs. Fluence rate was determined by integrating the 675–800 nm region of the spectral fluence rate curves measured with a spectroradiometer (ISCO, USA, model SR). All manipulations of seeds were conducted under a dim green safelight. Both the safe light and the R light source have been described previously (Georghiou and Thanos 1983).

Results

Skotodormancy as a function of temperature

The lettuce seed lot used in this work showed a remarkably low germinability in darkness throughout the temperature range $10\text{--}30^{\circ}\text{C}$ (Table 1). This rather unusual

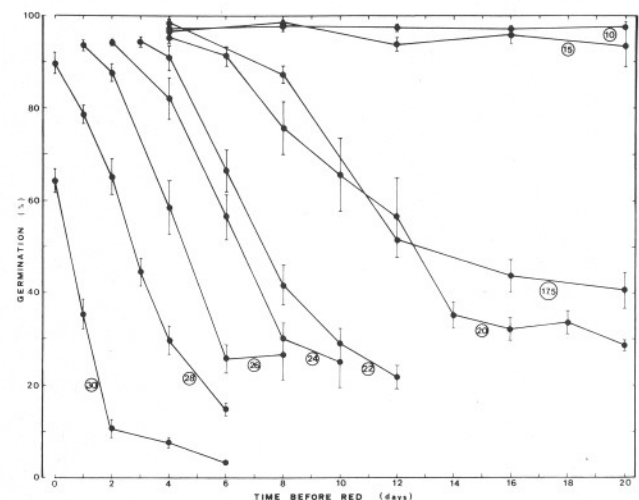


Fig. 1: The sensitivity curves to R at different temperatures (indicated by the number at each curve). Grand Rapids lettuce seeds were imbibed for various intervals in darkness and then illuminated for 10 min with Red light. Final germination was scored after various additional periods in darkness. Vertical bars = \pm SE.

property has permitted the experimentation on skotodormancy induction at low temperatures where no R light is usually needed for the promotion of germination. Fig. 1 presents the skotodormancy induction kinetics, i.e. the sensitivity curves to R as a function of duration of the imbibition pre-irradiation period, at different temperatures. It is clearly shown that skotodormancy was induced at a temperature equal or higher than 17.5 °C. Moreover, the time required for skotodormancy induction was temperature dependent: the lower the temperature the longer the period of imbibition required for skotodormancy induction. Thus, for half induction of skotodormancy at 20 °C a 10-fold time period (10.8 days) is needed compared with 30 °C (1.1 days). On the other hand, at 10 and 15 °C skotodormancy was not induced the R pulse being fully effective in promoting germination for at least the 20-day incubation period tested. Furthermore, when the time needed for half induction of skotodormancy was drawn as a function of temperature, the regression curve seems to follow either a linear or a negatively exponential curve (Fig. 2).

Photodormancy as a function of cFR fluence rate

In Fig. 3 the sensitivity curves to R in previously dark- and cFR-imbibed seeds as a function of the duration of pre-irradiation time are compared. The two extremes are (a) the induction of skotodormancy at 25 °C (previously dark-imbibed seeds) where a progressive loss of sensitivity to R was observed as in the preceding experiment, and (b) the persisting ability of R irradiation for full induction of germination in previously cFR imbibed seeds under the highest fluence rate used (1.6 W · m⁻²). Seeds imbibed under cFR of lower fluence rate values (0.8 and 0.4 W · m⁻²) presented an initial decrease in their responsiveness to R, tending to fol-

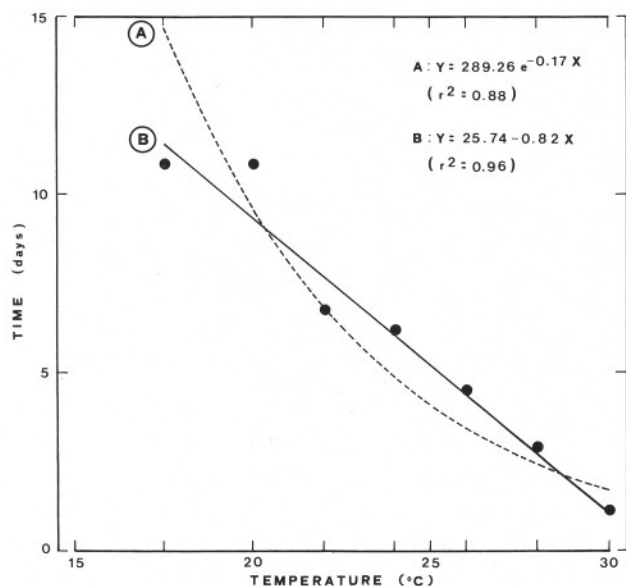


Fig. 2: Regression curves for the relationship between temperature and time of imbibition (preceding R) required for 50% inhibition of maximal germination (T_{50} values calculated from Fig. 1).

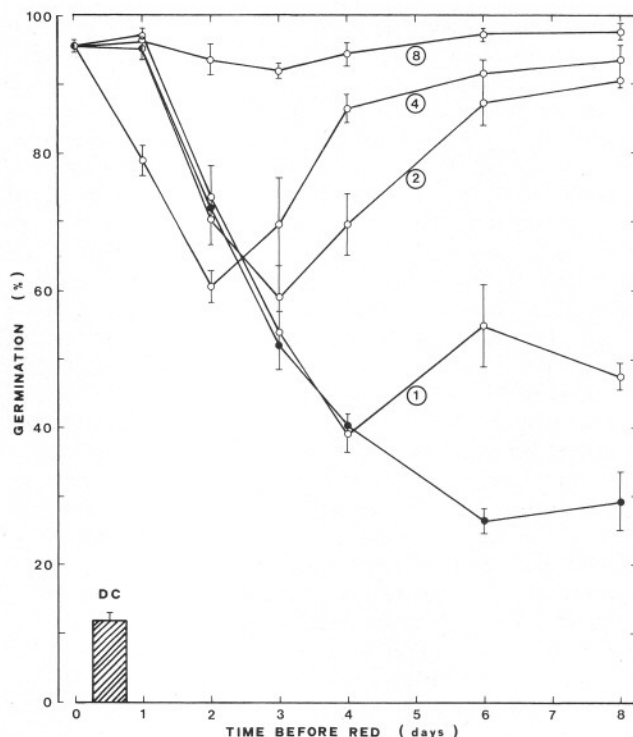


Fig. 3: The sensitivity curves to R in previously dark- and cFR-imbibed Grand Rapids lettuce seeds. Seeds were imbibed for various intervals in darkness (●) or under continuous Far Red irradiation (○) of 0.2, 0.4, 0.8 and 1.6 W · m⁻² fluence rate; the corresponding curves are indicated by the number of the lamps used (1, 2, 4 and 8 respectively). Final germination was scored after additional imbibition periods in darkness. Temperature was kept throughout at 25 °C. DC, dark control. Vertical bars = ±SE.

low the behaviour of previously dark-imbibed ones. However, a subsequent recovery for R promoted germination was observed, so that after 8 days of cFR exposure, R fully promoted germination. Even after much longer exposures under cFR of 0.8 W · m⁻² (12, 16 and 20 d) R is equally effective (data not shown). Under the lowest cFR fluence rate tested (0.2 W · m⁻²) the photosensitivity curve is identical to the corresponding dark one by the day 4. Subsequently, a statistically significant increase, compared to dark imbibed seeds, was observed. The onset of the recovery of photosensitivity, illustrated by the curves 1, 2 and 4 in Fig. 3, occurs earlier with increasing fluence rate, i.e. after 4, 3 and 2 days under 0.2, 0.4 and 0.8 W · m⁻², respectively.

FR pretreated and subsequently R irradiated seeds were generally slower germinators in comparison to not pretreated (primarily dormant). This is shown in Fig. 4. The time required for half of the final germination is 3 and 0.5 days respectively.

Discussion

The overwhelming majority of the Grand Rapids batches show a primary dormancy (relieved by light) only at rela-

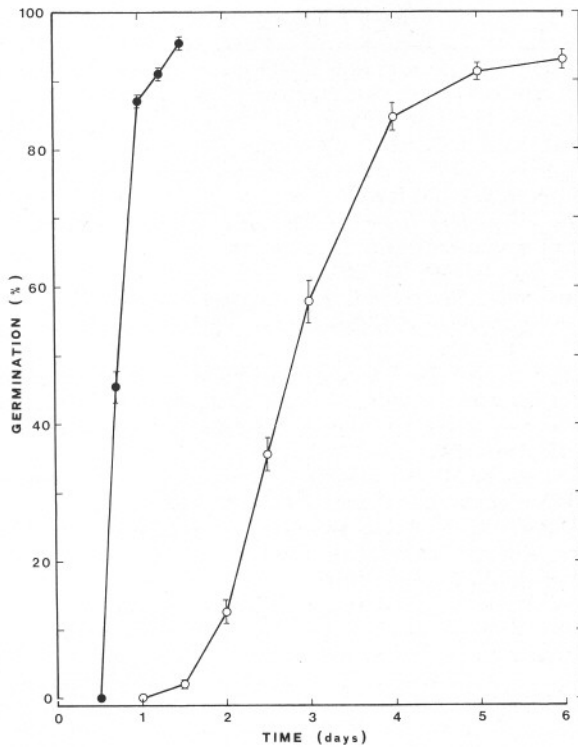


Fig. 4: The time-course of germination in darkness at 25°C of Grand Rapids lettuce seeds induced by R after 1 h of dark imbibition (●) or after 8 days of cFR ($0.8 \text{ W} \cdot \text{m}^{-2}$) irradiation (○) in the imbibed state. Vertical bars = \pm SE.

tively warm temperatures (usually above 20°C). The seed lot used in this work is obviously in a considerably deeper state of primary dormancy (Table 1). Between 10 and 30°C, dark germination never exceeded 30%, while a single R pulse, given shortly after the onset of imbibition, fully promoted germination (i.e. at least 90%) throughout the temperature range 10–28°C (Fig. 1). Thus the availability of this batch enabled the investigation of the temperature effect on skotodormancy induction. The results obtained (Fig. 1) show a clear temperature dependence for the range 17.5–30°C and are in agreement with those reported previously for other species. At the lower temperatures, 10 and 15°C, lettuce seeds remained responsive to R for the whole period tested (i.e. 20 days). This failure of seeds to enter skotodormancy has been also observed for *Rumex crispus* imbibed at 5°C (Le Deunff and Chaussat 1968, Taylorson and Hendricks 1973). But when *R. crispus* and *R. obtusifolius* were examined over considerably longer periods it was found that skotodormancy could be induced at temperatures as low as 1.5°C (Totterdell and Roberts 1979). According to Roberts and Totterdell (1981) there exist two opposing factors in *Rumex* seeds, a temperature-independent chilling effect on loss of primary dormancy (occurring below some critical upper temperature limit of about 15°C) and a temperature-dependent induction of secondary dormancy. In addition, the apparent transition of lettuce seeds from irresponsiveness to R responsivity, observed around 17°C (Fig.

1), is closely matched to the findings of VanDerWoude and Toole (1980) which indicate that prechilling temperatures below 18°C greatly enhance the photosensitivity of germination probably through the involvement of a membrane transition occurring near this temperature (Hendricks and Taylorson 1979). A further striking analogy is provided by the sharp threshold, near 17°C, displayed in the low-temperature enhancement of germination rate in dormant barley seeds (Roberts and Smith 1977).

The regression analysis of the kinetics of skotodormancy induction as a function of temperature has led to two alternative, regression curves (Fig. 2). When both these curves are extrapolated towards lower temperatures, the corresponding values for 15°C are estimated to 22.5 and 13.5 days, for curves A and B respectively. However, the R responsivity curve at 15°C did not show any sign of decrease, even after 20 days of dark imbibition (Fig. 1). This fact is in favour of the postulation of a critical temperature (between 17.5 and 15°C) below which skotodormancy cannot be induced; nevertheless, additional data with longer preirradiation periods are certainly needed.

According to the model proposed by Duke et al. (1977), seed germination is initiated after a sufficient level of interaction between P_{fr} and a «reaction partner» X is attained. Therefore, final germination levels are thought to be determined by the availability of both X and P_{fr} . In order to explain the skotodormancy induction data (Fig. 1) it is assumed that functional levels of X decrease during dark imbibition, the rate of decrease being temperature dependent. Since total phytochrome is considered to remain constant during dark imbibition, the loss of responsiveness to a R pulse, illustrated by the curves of Fig. 2, could reflect the decrease of the postulated functional levels of X. The induction of germination in skotodormant lettuce seeds requires a rather extended period of P_{fr} presence, achieved both by continuous and intermittent R irradiation (Georghiu and Thanos 1983). On the basis of the above mentioned model, this requirement might be attributed to the considerably decreased level of X. At lower temperatures the reaction partner X is maintained at levels high enough that skotodormancy fails to be imposed on lettuce seeds imbibed at 15°C or below, at least for the period of 20 days tested. The opposite effect of high temperatures (i.e. the induction of thermodormancy) could be similarly explained on the ground of thermal disappearance of partner X.

It is striking that whatever happens during dark imbibition, at 25°C, resulting eventually in the induction of skotodormancy, it did not occur when darkness during the preirradiation period was substituted by high fluence rate cFR (Fig. 3). This «protective» cFR action is obviously fluence rate dependent, so that with the lowest value used ($0.2 \text{ W} \cdot \text{m}^{-2}$) a skotodormancy induction curve, running very close to that of darkness, was obtained. This result could be explained by a decrease of steady-state P_{fr}/P_{tot} caused by decreased fluence rates under which thermal reversion of P_{fr} to P_r becomes significant relative to photochemical conversion of P_r to P_{fr} (Bartley and Frankland 1982, Jabben et al. 1982). According to those findings, higher light fluence rates should promote the formation of P_{fr} concentrations, in our case higher than those produced in darkness and

under low fluence rates. Therefore, certain P_{fr} fraction is expected to interact with partner X under cFR, the extent of this fraction being determined by fluence rate. Since cFR pretreated seeds never germinated without a R pulse before being transferred to darkness, it might be concluded that the interaction of P_{fr} and X is a prerequisite for the manifestation of the promoting effect of R.

The time-course of germination in the «far-red dormant» seeds was markedly delayed compared to that of the untreated, primary dormant ones. This fact has been observed previously for thermodormant and photodormant (Blaauw-Jansen 1981) as well as for skotodormant lettuce seeds (Georghiou and Thanos 1983). Although seed germination could be successfully reinduced in all cases, it is evident that an irreversible effect persists, the trace of which was revealed by the decrease of germination rate (Fig. 4).

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