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Phytochrome control of skotodormancy release in Grand Rapids lettuce achenes

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Light-requiring Grand Rapids lettuce (*Lactuca sativa* L.) achenes develop skotodormancy when imbibed in darkness for 7 days at 25°C. Redried skotodormant achenes maintain this type of dormancy upon subsequent rehydration. At 25°C full germination of skotodormant achenes can be induced by continuous and intermittent red light illumination as well as by several brief red irradiations given daily. One brief (10 min) red light irradiation can partly break skotodormancy at 20°C, while at lower temperatures the same treatment results in full induction of germination. Phytochrome control of the release from skotodormancy is proven by a) the dependence of the germination response on the relative sequence of red and far-red light in cyclic irradiations, and b) the reversion of red action by subsequent far-red irradiation. The time course of germination of skotodormant achenes treated with intermittent red light depends upon the length of dark interval between the light pulses. Germination is considerably delayed compared to that of non-skotodormant ones, induced by a single brief red light treatment. This fact in combination with the requirement, over a long period of time, of P_{fr} action for full manifestation of germination, indicates that skotodormancy is a deeper form of dormancy. It is concluded that the germination of lettuce achenes may always be subjected to phytochrome control.

Additional key words – Lactuca sativa, seed germination, temperature effect.

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Introduction

The failure of light-requiring lettuce achenes to germinate in response to white or red (R) light given after a prolonged period of dark imbibition is a well established phenomenon (Shuck 1936, Nutile 1943/44, Evenari and Neumann 1953, Borthwick et al. 1954, Ikuma and Thimann 1964). This inability is considered as a form of secondary dormancy and has been termed skotodormancy (reviews by: Barton 1965, Evenari 1965). The induction of skotodormancy has also been reported for many other light-requiring seeds such as *Chloris ciliata* (Gassner 1910), *Epilobium hirsutum* (Fassbender 1925), *Chenopodium album* (Karssen 1970), *Rumex crispus* (Taylorson and Hendricks 1973), *Sinapis arvensis* (Frankland 1976) and *Portulaca oleracea* (Duke et al. 1977).

Though skotodormancy generally refers to the ineffectiveness of light, it also includes other inductive agents. When lettuce achenes are immersed in gibberellic acid (GA) or benzyladenine (BA) after increased periods of dark imbibition in water, the induction of germination is gradually decreased (Ikuma and Thimann 1960, Vidaver and Hsiao 1974, Bewley 1980). It is worth noting that loss of responsiveness to both GA and BA is considerably quicker than to R light. Though each agent applied separately cannot promote germination of skotodormant achenes, the combination of R + GA at 20°C after 10 days of imbibition (Vidaver and Hsiao 1974), as well as R + BA

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at 23°C after 7 days (Bewley 1980), can fully induce germination. Although the achenes were not redried between induction and release of skotodormancy, the available data about seed desiccation lead to the conclusion that an intervening dehydration would not have any influence on the results mentioned above. In the only case where dehydrated skotodormant lettuce achenes were studied, R and GA, separately applied, were completely ineffective while endosperm removal resulted in full induction of germination (Bewley 1980).

The removal of the lettuce endosperm is well known to substitute for light requirement (Evenari 1965). This fact in combination with the synergistic promotive action of R and GA or BA has led us to investigate the control by light and phytochrome on the breaking of skotodormancy.

Abbreviations – BA, benzyladenine; c, continuous; D, darkness; FR, far-red; GA, gibberellic acid; i, intermittent; P_{fr}, far-red absorbing form of phytochrome; P_r, red absorbing form of phytochrome; R, red.

Materials and methods

Seeds and germination conditions

Light-requiring lettuce achenes (*Lactuca sativa* L. cv. Grand Rapids), 1978 harvest, were purchased from Carolina Biological Supply Co. (Burlington, NC, USA) and stored at 3°C. Induction of skotodormancy and germination tests were performed with 50 achenes per petri dish (diam. 7 cm) each lined with two layers of filter paper moistened with 3 ml of deionised water.

Skotodormancy was induced by a prolonged period of dark imbibition at 25°C, and germination was carried out at 3–25°C in temperature-controlled chambers. In all cases the temperature was kept constant within ±1°C. Ungerminated skotodormant achenes were air-dried for 48 h in darkness and 25°C, where they remained for not more than one week, until they were subsequently used in further experiments.

The criterion of germination was radicle protrusion and the tests were considered finished when no more germination occurred. Germination values are means of at least 5 replicates. Vertical lines and ± values represent standard error.

Light sources

R light (625–700 nm, emission maximum at 660 nm, 3 W m^{-2}) was obtained from 10 red fluorescent tubes (Philips TL 20 W/15) and one red 501, 3 mm thick, plexiglass filter. FR light (700–800 nm, emission maximum at 750 nm, 5 W m^{-2}) was obtained from 10 white incandescent tubes (Philips philinea 6276X60 W), one red 501 and two blue 627 plexiglass filters each 3 mm thick, and a 10 cm deep water bath. All manipulations of achenes were conducted under a green safelight (525–575 nm, emission maximum at 550 nm, 10 mW m^{-2}) obtained from a green fluorescent tube (F 15 T8·6 15 W Green-Photo, General Electric) filtered through one red orange 478 and one green 700 filter, each 3 mm thick. All plexiglass filters were purchased from Röhm GMBH, Darmstadt, W. Germany.

The emission spectra of the light sources were determined with an ISCO, model SR, spectroradiometer. Light intensities (at seed level) were measured with a YSI-Kettering, model 65, radiometer.

Brief (10 min) R and FR irradiations were given 1 h after onset of imbibition. In cases where temperatures lower than 25°C were used (Tab. 4), the achenes were imbibed for the first hour at 25°C, and then treated at the temperature indicated.

Results

Skotodormancy induction

Skotodormancy is fully induced after about 7 days of imbibition in complete darkness (Fig. 1). It is also observed that an initial FR irradiation, although reducing the percentage of germinating achenes, does not influence the kinetics of skotodormancy induction. The following experiments were carried out with achenes that were irradiated with FR, imbibed in darkness for 7 days and then dehydrated.

Release of skotodormancy by cR and iR

Although brief R irradiation was ineffective, the germination of skotodormant achenes was promoted by both continuous and intermittent R illumination (Tab. 1).

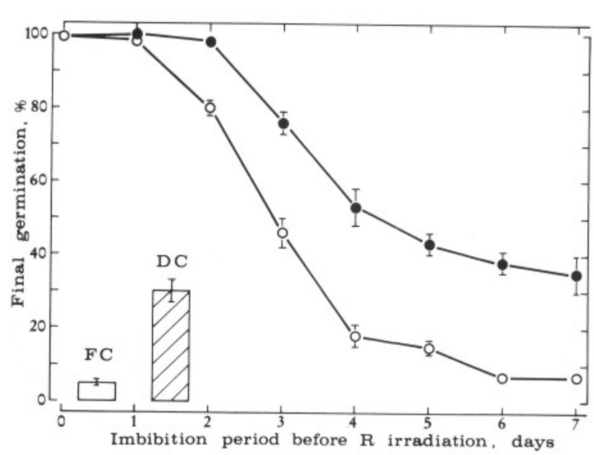

Fig. 1. The induction of skotodormancy at 25°C, with (○) and without (●) an initial FR irradiation. Achenes were imbibed for various intervals and then germination was induced by irradiating for 10 min with R light. Final germination was determined 72 h after the R light treatment. F C, far-red control; D C, dark control.

Tab. 1. The effect of various light treatments on the release of skotodormancy at 25°C. Final germination was determined 5 days after onset of imbibition. Intermittent light regimes were applied throughout the experiment with 1 min of red light given every 1, 4, 8, or 16 h. D, darkness. Values ± SE.

Light treatment	Germination (%)
D	0±0
R (10 min)	12±3
cR	95±1
iR (1 min/1 h)	98±1
iR (1 min/4 h)	99±1
iR (1 min/8 h)	98±1
iR (1 min/16 h)	94±1
i [R+FR] (1 min R + 1 min FR/1 h)	0±0
i [FR+R] (1 min FR + 1 min R/1 h)	98±1

The reversibility of the iR action by brief FR irradiations, given immediately after each R, clearly shows that breakage of skotodormancy is under phytochrome control.

Although all iR treatments tested resulted in full induction of germination (Tab. 1), Fig. 2 reveals a significant delay (about 6 h) in the time course of less frequently irradiated skotodormant achenes. It is also remarkable that germination of non-skotodormant achenes (induced by brief R) occurred completely before any germination of skotodormant achenes was observed.

Promotion of germination by cR (Tab. 1) can be only partly fullfilled by a long (12–24 h) R irradiation period (Tab. 2). In contrast, several (3–4) brief daily R irradiations can fully break skotodormancy (Tab. 3).

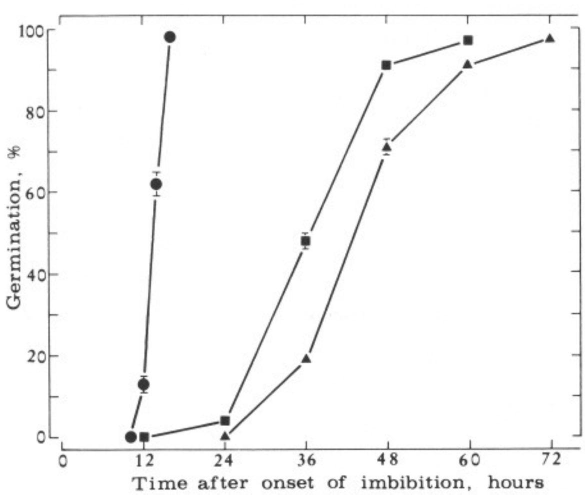

Fig. 2. Time course of germination of lettuce achenes at 25°C. ●, untreated (non-skotodormant) irradiated with a brief (10 min) initial R; ■, ▲, skotodormant achenes irradiated with iR (1 min R/1 h and 1 min R/8 h, respectively).

Tab. 2. The effect of different periods of R irradiation, given 1 h after onset of imbibition, on the release of skotodormancy at 25°C. Final germination was determined 5 days after sowing. Values ± SE.

Irradiation period (h)	Germination (%)
0	0±0
1	20±6
2	36±3
4	56±8
6	66±4
12	82±4
24	87±3

Tab. 3. Final germination of skotodormant achenes at 25°C, given various numbers of brief (10 min) R irradiations (separated by 24 h dark periods), starting 1 h after onset of imbibition. Germination was scored 4 days after last irradiation. D, darkness. Values ± SE.

Light treatment	Germination (%)
D	0±0
1×R	10±3
2×R	74±3
3×R	92±2
4×R	96±1

Release of skotodormancy by brief R

In the previous experiments which were performed at 25°C, it was shown that relief from skotodormancy was achieved by cR and iR as well as by several brief R irradiations given daily. In order to reduce the light requirement to a single brief R irradiation, subsequent experiments were carried out at lower temperatures. It is well documented that dark germination of typical Grand Rapids lettuce achenes is favoured at low temperatures. This was also confirmed for the untreated

Tab. 4. The effect of a single brief (10 min) R irradiation on the release of skotodormancy as a function of temperature. Corresponding data for untreated achenes are included for comparison. Final germination was determined 5 days (at 25, 20 and 15°C) and 16 days (at 10 and 3°C) after onset of imbibition. D, darkness. Values ± SE.

Temperature (°C)	Germination (%) of skotodormant achenes			Germination (%) of untreated (non-skotodormant) achenes		
	D	R	R+FR	D	FR	FR+R
25	0±0	5±1	–	26±4	6±1	99±1
20	0±0	66±3	–	50±3	17±2	100±0
15	0±0	98±1	0±0	76±2	41±4	100±0
10	0±0	98±1	–	40±4	10±2	99±1
3	0±0	99±1	–	94±2	64±3	100±0

achenes used in this study (Tab. 4). In contrast, dark germination of skotodormant achenes was not apparently promoted, even after 16 days at 3°C. The most important result is the fully promotive action of brief R at 3–15°C and the considerable increase of germination even at 20°C. The complete reversal by brief FR shows again the involvement of phytochrome.

The efficiency of R light for induction of germination was further tested with skotodormant achenes which either had been irradiated initially with FR and imbibed in darkness for 14 days, or imbibed for 7 days but without the initial FR irradiation. The data obtained in these experiments are quite similar to the results presented for 7-day (+ initial FR) skotodormant achenes (Tabs 1 and 4).

Discussion

Skotodormancy (Bewley 1980) as well as other types of secondary dormancy (K. Georghiou, unpublished data) in lettuce achenes is not affected by dehydration following the inductive treatment. This was also true in this study (compare R actions in Fig. 1 and Tab. 1), allowing experimentation with redried skotodormant achenes. In order to avoid the probable loss of dormancy due to dry storage (observed in many cases, e.g. Suzuki et al. 1980), the experiments on skotodormancy release were carried out within a few days after desiccation.

The similar kinetics for skotodormancy induction in both dark- and brief FR-treated achenes are in agreement with the corresponding results by Vidaver and Hsiao (1974). Moreover, the release of skotodormancy is not influenced by initial brief FR irradiation.

The most important finding of this study is the release of skotodormancy by light. At 25°C, both continuous and intermittent R irradiation, can fully induce germination of skotodormant achenes. At lower temperatures, even one brief R irradiation is equally effective. The involvement of phytochrome in breaking skotodormancy is clearly shown by the R/FR reversibility obtained in both brief and intermittent irradiation regimes. Therefore, the postulation proposed by Smith (1975), that phytochrome itself might be again the key, seems justified and the two alternative explanations suggested, namely the diminished presence of either (a) P_r or (b) a substance combining with P_{fr}, seem equally probable at the moment.

Since, in prior studies (Shuck 1936, Bewley 1980), skotodormancy could not be relieved by either R light or growth regulators alone, it has been considered as a deeper state of dormancy, different than the primary one. Our results confirm the concept that skotodormancy is really a deeper form of dormancy, but this conclusion is based on different reasons: (a) phytochrome action for germination induction of skotodormant achenes, at 25°C, is required over a long period of time; (b) the corresponding time courses of germination are considerably delayed; and (c) no dark germination occurs, even at low temperatures (3–15°C).

It is furthermore proposed that skotodormancy of lettuce achenes, though it appears to be a deeper state of dormancy, is of a nature similar to the primary as well as other forms of secondary dormancy. This suggestion is based on the following data: (a) phytochrome activation induces germination; (b) light requirement decreases with lower temperatures; (c) brief R together with GA or BA can break skotodormancy (Vidaver and Hsiao 1974, Bewley 1980); and (d) isolated embryos from skotodormant achenes germinate in darkness (Bewley 1980). The only contradictory arguments are the ineffectiveness of growth regulators and chilling. Nevertheless, for their definite exclusion as breaking agents of skotodormancy, a more detailed investigation, particularly on dehydrated achenes, is certainly needed.

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