THE LIGHT PROMOTED GERMINATION OF THE SEEDS OF CHENOPODIUM ALBUM L. VI. Pfr REQUIREMENT DURING DIFFERENT STAGES OF THE GERMINATION PROCESS

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SUMMARY

Positive photoblastic seeds of *Chenopodium album* need the action of the active form (Pfr) of phytochrome (P) during nearly the total length of the overall germination process. The first Pfr dependent processes require a Pfr/P ratio above the level being present in darkness. The different requirement for the length of the red irradiation indicates that these first processes have a different Pfr requirement in the individual seeds of one population. Simultaneous irradiations with narrow wavelength bands of red (643 nm) and far-red (768 nm) light, which established different Pfr/P ratios, supported this conclusion. The Pfr requirement is a function of the thickness of the seed-coat.

The germination of half operated seeds (the outer seed-coat layer is removed in the area overlying the radicle) can be induced by the dark level of Pfr, being approximately Pfr/P = 0.07.

The required Pfr level for the germination processes after the escape from the antagonistic effect of short far-red irradiations, decreases from 0.07 to below 0.02. Osmotic stress increases the Pfr requirement. Prolonged irradiations with far-red light inhibit the germination processes when they are already in the phase of the embryo elongation. The elongation process is supposed to consist mainly of cellular expansion. The process which induces the secondary photodormancy is only related to the first Pfr dependent processes.

1. INTRODUCTION

The recent estimations of phytochrome in seeds have confirmed the previous assumption that seeds incubated in darkness can maintain a fraction of the total phytochrome content in the far-red absorbing form (Pfr) (see KARSSEN 1970b for references). Whereas this dark level of Pfr can promote the germination of non-photoblastic seeds, positive photoblastic seeds evidently need a higher Pfr level.

We reported in our previous paper (KARSSEN 1970c) that in positive photoblastic seeds of *Chenopodium album* about 48 hours after the inducing red irradiation, the germination processes can no more be prevented by a short far-red irradiation. It is a well-known fact that far-red light converts nearly all Pfr to Pr. When the inverse dark reversion reaction (BOISARD *et al.* 1968) can also proceed in the seeds of *C. album*, as was supposed before (KARSSEN 1970b), the Pfr level will return to the dark level after the far-red irradiation. MOHR & APPUHN (1963) found in lettuce seeds that prolonged irradiations with far-red light have still an inhibitory effect after the escape from short irradiations. It

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seems therefore that Pfr is still required during the later phases of the germination process, albeit at a much lower level than before.

In the present study we will deal with two aspects of the course of the Pfr requirement during the subsequent phases of the overal germination process in positive photoblastic seeds of *C. album*. In the first place we will investigate in more detail the different requirement for red light. In preliminary experiments it was observed that in these seeds this can vary from an irradiation during some seconds to a continuous one. The neeo for a continuous or intermittend irradiation with red light has often been observed in several other species. Whereas lettuce seeds (var. Grand Rapids) need red light for only a few seconds or minutes (BORTHWICK *et al.* 1954), *Plantago* seeds must be irradiated for 1 hour, *Epilobium* seeds for 24 hours (ISIKAWA & YOKOHAMA 1962) and *Paulownia* seeds for 48 hours, either continuously or intermittendly (TOOLE 1961; BORTHWICK *et al.* 1964). We will investigate whether the gradation in need for red light is an indication for a different Pfr requirement of the individual seeds of one population of *C. album* seeds.

A second aspect of the present study will be the Pfr requirement of the processes after the escape reaction. It will be investigated by means of prolonged irradiation with far-red light whether the small level of Pfr, presumably being present during the later phases of the processes, has still a function in *C. album* seeds. Moreover the dependency of the Pfr requirement on conditions of osmotic stress, which was observed in non-photoblastic seeds (KARSSEN 1970b), will be investigated in these positive photoblastic seeds.

2. MATERIAL AND METHODS

All experiments of the present study were done with the same selection of seeds as used in our previous study (KARSSEN 1970c).

Nearly all methods employed in the present experiments have been described before. See KARSSEN (1967, 1968) for germination and storing conditions and for a description of the green "safelight" source; KARSSEN (1970a) for a description of the operation technique and the determination of the seed-coat thickness; KARSSEN (1970b) for the method of incubation in mannitol solutions, a description of the stages of the visible germination phenomena and the far-red light source (intensity 50 μWatt.cm^{-2} between 700 and 800 nm); KARSSEN (1970c) for the red light source (intensity 200 μWatt.cm^{-2}).

To obtain various photostationary states of phytochrome (ratio between the far-red absorbing form and total phytochrome: Pfr/P) the seeds were in some experiments irradiated simultaneously with red light (643 nm) and far-red light (768 nm). The source of either being an incandescent (iodine) lamp collimated by an appropriate system of lenses and a 5 cm layer of water in combination with either a 643 nm DEPAL double band filter (Schott & Gen., Mainz, W-Germany) and a Calflex-C filter (Balzer, Liechtenstein) or a 768 nm DEPAL filter. The intensities were approximately 140 μWatt. cm^{-2} and 100 μWatt. cm^{-2},

respectively. The intensities of both light sources could be regulated independently by means of two variable transformers (Variac).

The photostationary states, which were established by a combination of both light sources, were calculated by means of an equation, according to SIEGELMAN & BUTLER (1965) and HARTMANN (1966):

$$\frac{Pfr}{P} = \frac{1}{1 + \dfrac{\alpha_{fr.\lambda 1} . \varnothing_{fr} . I_{\lambda 1} + \alpha_{fr.\lambda 2} . \varnothing_{fr} . I_{\lambda 2}}{\alpha_{r.\lambda 1} . \varnothing_{r} . I_{\lambda 1} + \alpha_{r.\lambda 2} . \varnothing_{r} . I_{\lambda 2}}}$$

where $\alpha_{r.\lambda}$ and $\alpha_{fr.\lambda}$ are the molar-extinction coefficients (liter mole^{-1}cm^{-1}) of Pr and Pfr at λ, $\varnothing_r$ and $\varnothing_{fr}$ are the quantum yields (moles Einstein^{-1}) for the photochemical conversions of Pr and Pfr and I_λ is the intensity (Einstein cm^{-2}. sec^{-1}). The products of $\alpha . \varnothing$ for both pigment forms and at both wavelengths were obtained from the action spectra of photochemical transformations of Pr and Pfr (SIEGELMAN & BUTLER 1965).

A correction was necessary to eliminate the differences in transmission of the seed-coat layers at both wavelengths. The ratio between the optical densities of these layers at 643 and 768 nm was approximately 5 to 1.

3. RESULTS

3.1. Red light requirement

The effects of red irradiations, differing in duration from 25 sec to 192 hours, on the germination of a selection of *Chenopodium album* seeds is represented in *fig. 1.* VAN ROODEN *et al.* (1970) obtained rather similar results with seeds of *Portu-*

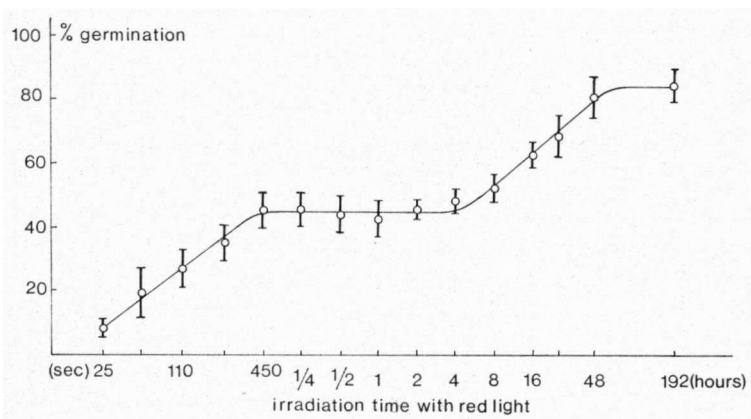

Fig. 1. The relation between the irradiation time with red light (plotted on a logarithmic scale) and the germination response. All irradiations started after an incubation of 24 hours in darkness. The vertical bars represent the standard deviations of the mean. The points are the mean of 4 to 20 experiments with 2 dishes each.

laca oleracea. The increase of the germination response after irradiations up to 450 sec is most probably due to an increasing saturation of the photochemical conversion of the red absorbing form of phytochrome (Pr) into the far-red absorbing form (Pfr).

A saturating irradiation of 450 sec induces germination in only 45% of the seeds. To obtain a higher percentage the duration of the irradiation has to be 8 hours or more. A continuous irradiation of 48 hours can be replaced by intermittent irradiations of 15 min either in every hour or in every 8 hours during the same period (*table 1*). Even three irradiations of 15 min, after respectively 24, 48 and 72 hours of dark incubation, cause significantly more germination, when compared with single irradiations. Two irradiations of 15 min, at the beginning and the end of a certain period, can almost completely replace a continuous irradiation, when the interval is not longer than 24 hours (*table 2*). When it is longer the second irradiation gradually loses its promoting effect (*fig. 2*).

Table 1. The effects of continuous or intermittent irradiations with red light during a period of 48 hours. The irradiations started after 24 hours of dark incubation

Type of irradiation	Total irradiation time	% S.D.	n
continuous	48 hours	80.3 ± 6.0	20
intermittent 15 min/ 1 hour	12 hours	78	2
intermittent 15 min/ 8 hours	105 min	78	2
intermittent 15 min/16 hours	60 min	72	2
intermittent 15 min/24 hours	45 min	68.8 ± 5.3	24
controls:			
15 min after 24 hour		45.9 ± 5.7	50
15 min after 48 hour		48.6 ± 6.5	28
15 min after 72 hour		39.8 ± 6.0	6

%: percentage germination
SD: standard deviation of the mean
n: number of dishes (two per experiment)

Table 2. A comparison between the effects of a continuous irradiation with red light during a certain period and the effects of two red irradiations of 15 min at the beginning and the end of the same period.

Incubation period (hours after start of incubation)	Continuous red light		15 min red light at beginning and end	
	% S.D.	n	% S.D.	n
24 to 32 hours	52.2 ± 4.5	4	48.5	2
24 to 40 hours	62.6 ± 3.1	8	57.5	2
24 to 48 hours	68.9 ± 6.8	12	62.2 ± 2.8	10
24 to 72 hours	80.3 ± 6.0	20	55.5 ± 6.6	4

%: percentage germination
SD: standard deviation of the mean
n: number of dishes (two per experiment)

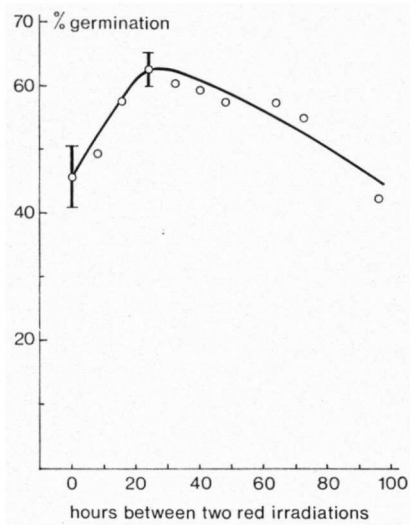

Fig. 2. The effect of a second red irradiation of 15 min given at a variable time after a first red irradiation (after 24 hours of dark incubation). The vertical bars represent the standard deviations of the mean.

It appears from these results that a Pfr level of 0.81 (established by a saturating red irradiation, BUTLER *et al.* 1964) must be present, continuously or intermittently, for at least 8 hours to obtain more than 45% germination. These results support our previous assumption (KARSSEN 1970c) that in *C. album* seeds Pfr can be reverted to Pr in darkness. BORTHWICK *et al.* (1964) draw the same conclusion from rather similar observations in *Paulownia* seeds.

The duration of the red irradiation does neither influence the length of the escape reaction nor the rate of the processes which proceed afterwards (*fig.* 3). The antagonistic effect of a far-red irradiation of 30 min is always finished 48 hours after the beginning of the red irradiation. So it is evident that the processes requiring a Pfr level above the dark level always proceed within 48 hours. To obtain a germination response in 80% of the seeds it is necessary to irradiate the seeds with red light during the whole 48 hours period, either continuously or intermittently. A response of 45% is already obtained after one saturating irradiation of 15 min at the beginning of the period. The decrease of the Pfr level, which presumably takes place in the latter case, does evidently not prevent the progress of the first Pfr dependent processes in this fraction of the population. As also after a short irradiation the Pfr level can only be decreased to the dark level after 48 hours, it can be concluded that Pfr levels below 0.81 have still a regulating function.

In general these data support the conclusion that the individual seeds of the population need a different Pfr level for the first processes after the beginning of the red irradiation.

The same conclusion can be drawn from the effects of a range of different Pfr/P ratios, established for 48 hours by intermittent irradiations (10 min per hour) with a combination of red (643 nm) and far-red light (768 nm) (*fig.* 4). It

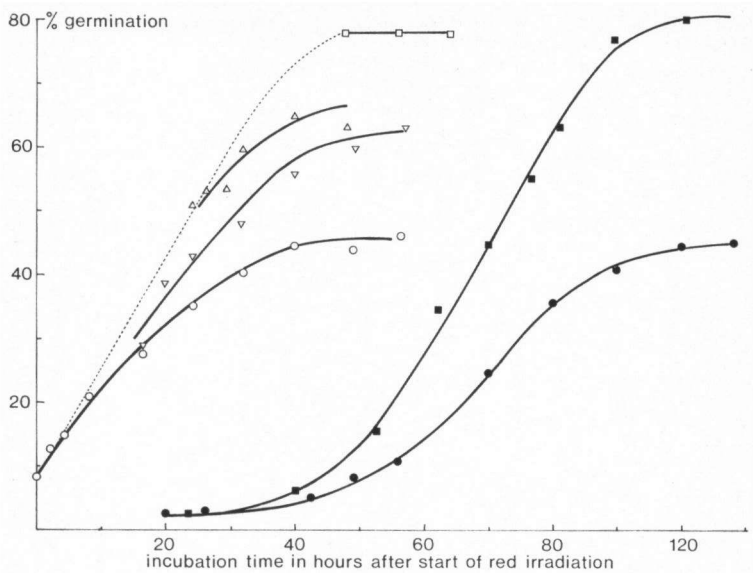

Fig. 3. Open symbols: the escape from the antagonistic effect of a short far-red irradiation (30 min) given at a variable time after the start of either a red irradiation of 15 min (○) or intermittent irradiations (15 min per hour) during 16 (▽), 24 (△) or 48 (□) hours. All red irradiations started after 24 hours of dark incubation.
Closed symbols: the time course of the germination process after either 15 min red light (●) or 48 hours (15 min/hour) red light (■).

can be seen that the Pfr level required for the promotion of the germination response varies from about 0.05 to 0.40. When the same Pfr/P ratios were established for only 15 min, and therefore afterwards could decrease again it appeared that the effect was much smaller. The rather similar effects of a Pfr/P ratio of 0.15, either established during 15 min or 48 hours, indicate that this level can be maintained in darkness. Higher Pfr levels evidently decrease too fast in darkness. Consequently only those seeds will germinate which have a requirement for a Pfr level lower than the one initially established.

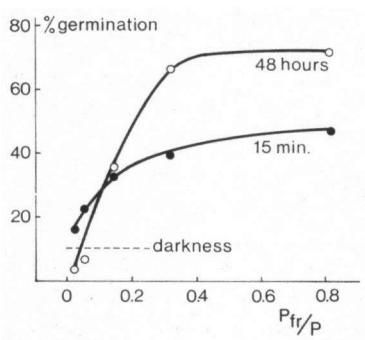

Fig. 4. The relation between the photostationary states (Pfr/P) established by simultaneous irradiations of red light (643 nm) and far-red light (768 nm) in different intensity combinations. The total intensity in all combinations was 100 μWatt.cm^{-2}. The irradiations were given either during 15 min (after 24 hours of dark incubation) or during 48 hours (10 min per hour from 24 to 72 hours after the start of the incubation).

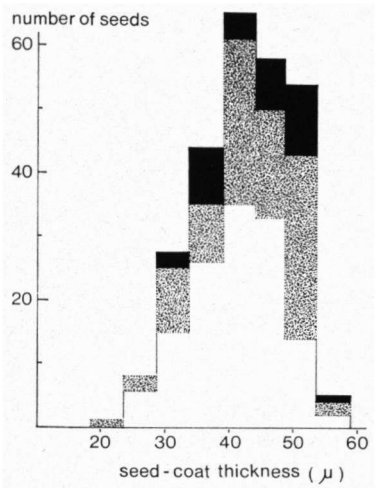

Fig. 5. The distribution of the seed-coat thickness of the germinated seeds out of groups of 300 seeds, which were irradiated with either 15 min or 48 hours red light, started after 24 hours of dark incubation. Open area: 15 min red light; open + spotted area: 48 hours red light; open + spotted + closed area: distribution in the total population of 300 seeds (germinated and ungerminated).

Our previous conclusion (KARSSEN 1970b) that the Pfr requirement of the seeds of *C. album* is determined, among other things, by the thickness of the seed-coat, is supported by the present results. The seeds which can only germinate after a red irradiation of 48 hours have for the greater part thicker seed-coats than the seeds which require only 15 min red light (*fig. 5*).

3.2. Pfr requirement of operated seeds

The relation between the Pfr requirement of the seeds and the seed-coat was also tested by means of the operation technique. It has been reported before (KARSSEN 1968, 1970a) that positive photoblastic seeds of *C. album* lose their light dependency either completely or a great deal, when respectively both seed-coat layers or only the outer layer are removed in the area overlying the radicle. The effect of continuous far-red light (*table 3*) shows that half-operated seeds require a Pfr level above 0.02 (established by the far-red light source (see below), whereas fully operated seeds have a still lower Pfr requirement. These data evidently also support our previous conclusion.

Table 3. Effects of red and far-red light on the germination of half operated seeds (outer seed-coat layer removed in the area overlying the radicle) or fully operated seeds (both seed-coat layer and the endosperm layer removed in that area).

	Percentages germination in continuous:		
	red light	far-red light	darkness
fully operated seeds	100	100	100
half operated seeds	96	10	90
unoperated seeds	82	2	10

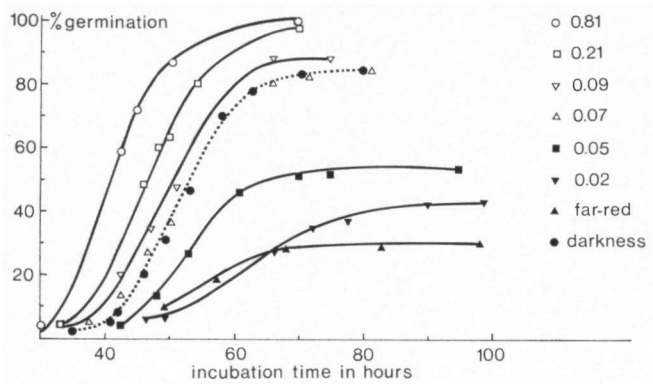

Fig. 6. The influence of the photostationary state (Pfr/P) during the first 24 hours of incubation on the germination time course and the final germination percentage of half operated seeds. See for the method of irradiation *fig. 3* and Methods. The far-red light (▲) was obtained from the source used in all the other experiments (see *e.g. fig. 7*).

By means of simultaneous irradiations with red (643 nm) and far-red light (768 nm) of different intensity combinations during the first 24 hours of incubation, the effects of different Pfr/P ratios on the time course and the final germination percentage were determined (*fig. 6*). It appeared that quantity and rate of the germination response were identical after both an incubation in darkness and the establishment of a Pfr/P ratio of 0.07 during the first 24 hours. It seems, therefore, reasonable to conclude that the seeds of *C. album*, used for the present experiments, can maintain this particular Pfr level in darkness.

An increase of the Pfr/P levels above 0.07–which is an absolute requirement for the germination of unoperated seeds (*fig. 4*) – accelerates the germination of half operated seeds (*fig. 6*) up to 12 hours (Pfr/P = 0.81). A short red irradiation (15 min) after 2 hours of incubation in darkness had the same effect. At the moment the first visible elongation processes can be observed in the half operated seeds (i.e. after about 16 hours of dark incubation), such an accelerating effect can no more be induced. It is suggested therefore that Pfr levels above 0.07 in half operated seeds increase the rate of the processes preceeding the elongation of the embryo.

Pfr/P ratios below 0.07 cause an inhibition of the germination response. A comparison between the effects of the simultaneous irradiations with red and far-red light and those of irradiations for the same period of time with far-red light from the source used in many present and previous experiments reveals that the source establishes a Pfr/P ratio of about 0.02 (*fig. 6*).

3.3. Pfr requirement after the escape reaction

Prolonged irradiations with far-red light can still inhibit the germination of *C. album* seeds when they have been escaped from the antagonistic effect of a short far-red irradiation (*fig. 7*). As we discussed before (KARSSEN 1970b) the

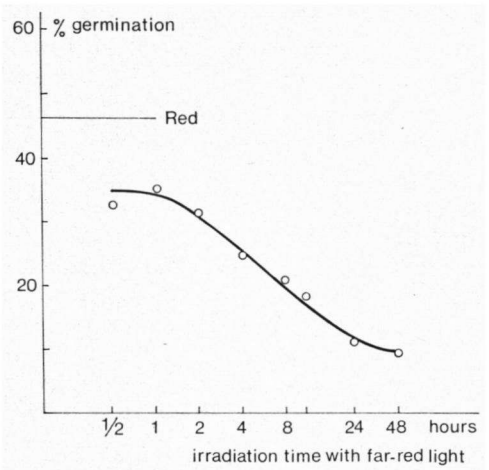

Fig. 7. The relation between the length of far-red irradiations (plotted on a logarithmic scale) and the inhibition of the effect of a short red irradiation (15 min). Red light was given after 24 hours of dark incubation, the far-red irradiations started 16 hours after red light.

effect of prolonged far-red irradiations is most obviously due to the continuous antagonizing of the effect of the inverse dark reversion reaction, which is able to reestablish the dark Pfr level. It can be concluded, therefore, that the seeds need, during the later phases of the processes, only a Pfr level between 0.07 and 0.02, being respectively the dark level (see 3.2.) and the level established by the far-red irradiation. To determine the Pfr requirement during the course of the later phases of the germination process, far-red irradiations of 24 hours were started at different moments after the red irradiation (15 min). The prolonged far-red irradiations could stop the processes till about 20 hours before the moment the radicles protrude through the inner seed-coat layer (*fig. 8*). Thirty minutes far-red

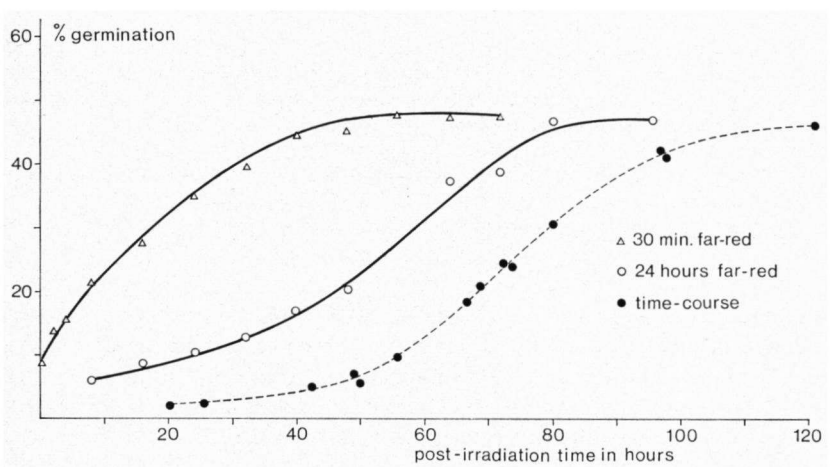

Fig. 8. The effects of far-red irradiations of either 30 min or 24 hours given at a variable time after a single red irradiation (15 min) on the final germination percentage. The dotted line represents the time course (stage III, see *fig. 9*) of the red light control (irradiated after 24 hours of dark incubation).

light can do so at latest 60 hours before that moment. During the last 20 hours of the germination processes the Pfr requirement seems to have decreased below 0.02.

An important aspect of the effects of prolonged far-red light is that it can still inhibit the processes when they are in the phase of the embryo elongation, which in *C. album* seeds becomes visible before the ultimate protrusion of the rootlet through the inner seed-coat layer (KARSSEN 1968). A far-red irradiation of 24 hours, started 48 hours after the first red irradiation (15 min on the 24th hour, followed by 15 min on the 48th hour), inhibits the process at all three stages (*fig. 9*). The last stage is inhibited, however, at a somewhat slower rate than the earlier ones.

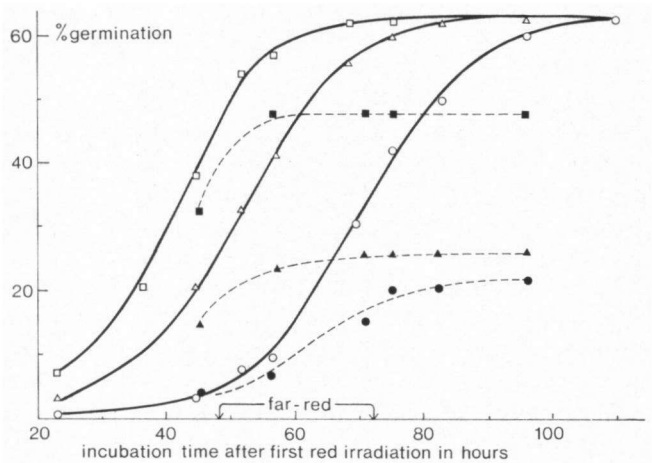

Fig. 9. The germination time courses of the three stages of the visible germination phenomena after two short red irradiations (15 min, 24 and 48 hours after the start of the incubation) either without (open symbols) or with (closed symbols) a far-red irradiation of 24 hours, started 48 hours after the first red irradiation.
□: stage I (splitted outer seed-coat layer);
△: stage II (rootlet extended from within the seed, but still enclosed by the inner-seed-coat layer);
○: stage III (radicale has protruded through inner seed-coat layer).

We assumed before (KARSSEN 1968) that the elongation process in the seeds of *C. album* consists mainly of cellular expansion, as was reported for several other species. Negbi (personal communications) found that in another species of the Chenopodiaceae (*Salsola volkensii*) cell division did not occur in the embryo and in the seedling till about 24 hours after the protrusion of the rootlet through the seed-coat.

An inhibitory effect of mannitol solutions on seed germination is often taken as a strong argument for the participation of cellular expansion in the rootlet protrusion (HABER & LUIPPOLD 1960). We reported before (KARSSEN 1970b)

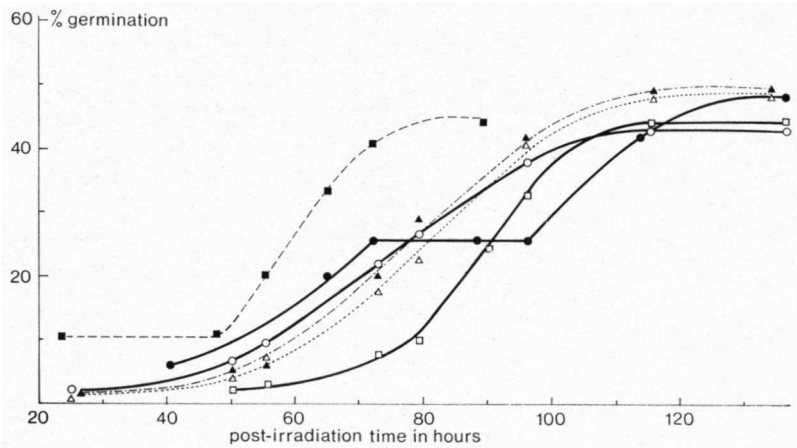

Fig. 10. The germination time courses of seeds irradiated with 15 min red light after 24 hours of dark incubation. The seeds were incubated during 24 hours of the incubation period in 0.6 M mannitol, the remaining time in water. The mannitol incubation took place during the 24 hours before red light (▲) or from 0 to 24 hours (△); 24 to 48 hours (□) or 48 to 72 hours (●) after red light. Water control (○). All curves represent stage III of the visible phenomena (see *fig. 9*), except the curve indicated with (■) (stage I of the seeds incubated in mannitol from 24 to 48 hours after red light).

that non-photoblastic seeds of *C. album* could be inhibited by higher osmotic concentrations. In the present experiments the inhibitory mannitol effect could be localized in the phases of visible elongation of the embryo. *Fig. 10* shows that an incubation during 24 hours in 0.6 M mannitol delayed the course of the germination processes only when started 24 or 48 hours after the red irradiation. The first seeds reach stage I after 24 hours. Before or immediately after red light no effect could be observed.

A comparison between *figs. 8* and *11* shows that a far-red irradiation of 24 hours can inhibit the germination processes also during the last 20 hours, when the seeds are transferred from water to 0.6 M mannitol at the moment the irradiation starts. In these experiments the seeds stayed in mannitol during the remaining part of the incubation time. Osmotic stress thus increases the Pfr requirement of the processes. Also during the last 20 hours Pfr must be present at a level above 0.02.

Just as in non-photoblastic ones in these seeds the Pfr requirement showed a progressive relation with the mannitol concentration. After a decrease of the Pfr level to 0.02, by means of far-red light at the 48th hour after a red irradiation, 21 % of the seeds germinated when they stayed in water, 14 % and 7 % when they were transferred to respectively 0.4 and 0.6 M mannitol for the rest of the incubation period.

A red irradiation of 15 min, given either immediately or 72 hours after an inhibitory far-red irradiation of 24 hours, immediately restarts the germination processes in the seeds which had already reached stage I of the visible germination

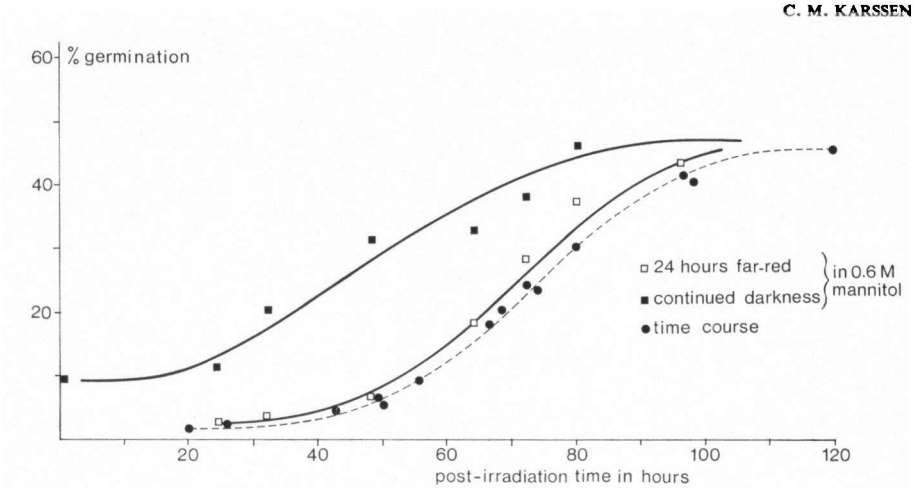

Fig. 11. The effects of an incubation in 0.6 M mannitol on the final germination percentages. The incubation started at a variable time after a short red irradiation (15 min after 24 hours of dark incubation) either in combination with 24 hours far-red light (□) or in continued darkness (■). The dotted line represents the time course of the red control (stage III, see *fig. 9*).

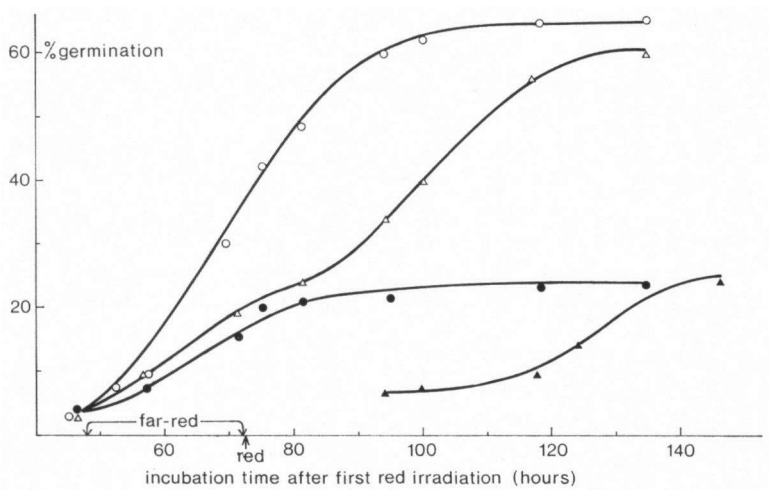

Fig. 12. The germination time course (stage III, see *fig. 9*) of seeds which received one of the following irradiations: 3 times 15 min red light (24, 48 and 72 hours after start of incubation) (3R) (○); 3R + 24 hours far-red light (72 to 96 hours) (FR) (●); 3R+FR + 15 min red light (on the 96th hour) (△); only 15 min red light after 96 hours of dark incubation (▲).

Table 4. Effects of short red irradiations (15 min) given after long periods of incubation. During the first 96 hours the seeds were kept either in darkness or irradiated with red and far-red light.

Treatment during the first 96 hours of incubation	Red irradiation after (hours):			
	96	120	144	168
Darkness	15	13	10	7
D24hr – R15min – D48hr – FR24hr*	38	35	38	35

* Without a subsequent red irradiation the percentages of seeds that reached the three stages were after this treatment: 38 (I), 25 (II) and 20 (III), see *fig. 9* for description of the stages. An irradiation with 15 min red light after 24 hours of dark incubation caused a germination response of 45% (*fig. 1*).

phenomena (*fig. 12, table 4*). Such a red irradiation had either no effect or only a small and much slower one in seeds which respectively had not yet reached stage I or had not been irradiated at all till that moment. It can be concluded, therefore, that the process, which causes the decrease of the reactivity of the seeds to red light, the so-called secondary photodormancy (KARSSEN 1967, 1970c) inhibits the effect of red light only when the elongation processes have not yet started.

4. DISCUSSION

The results presented in this paper allow the conclusion that phytochrome action is required during nearly the complete duration of the overall germination process in positive photoblastic seeds of *C. album*. During the subsequent phases of the process the seeds require, however, a different ratio between the active form of phytochrome and the total pigment content (Pfr/P). In seeds of several species a stable concentration of the germination regulating form of the pigment ('seed phytochrome') has been observed (see KARSSEN 1970b for references). If the seeds of *C. album* possess the same characteristic, a dependency of the germination on the Pfr/P ratio means in fact a dependency on the absolute concentration of Pfr.

In the red light dependent seeds two phases can be discerned in the Pfr requirement. During the first one the seeds need a Pfr level above the dark level. During the second phase they need maximally the presence of the dark level, being approximately 0.07 in these seeds (*fig. 6*).

In half or fully operated seeds the first phase of Pfr requirement is absent, just as in non-photoblastic seeds of *C. album* (KARSSEN 1970b). The latter seeds have rather thin seed-coats (KARSSEN 1970a). It seems, therefore, reasonable to conclude that the requirement for a Pfr level above the dark level depends on the presence of a thick seed-coat. The seeds of one lot of *C. album* seeds appear to have a different Pfr requirement during the first 48 hours after the beginning of the red irradiation (*fig. 4*). Also this gradation could be related to the seed-coat thickness (*fig. 5*).

Our present and previous reported results agree, therefore, with the conclusion of BLACK (1969), based on a review of recent literature, that the amount of Pfr which is required for the promotion of germination depends strongly on the conditions of stress imposed upon the embryo. Normally this stress is achieved by the tissues surrounding the embryo.

It is not known how the enclosing structures succeed in inhibiting embryo growth. Some authors suggest that it is primarily a mechanical effect (IKUMA & THIMANN 1963), others have proposed that inhibitors are involved (BLACK & WAREING 1959), while also an impermeability to gases can be regulating in the effect (BROWN 1965). Detailed information about the effect of the seed-coat in *C. album* seeds is absent. We can only refer to some of our previous experiments (KARSSEN 1968) which suggest that the inner seed-coat layer and the underlying endosperm layer form a certain mechanical restraint.

The present experiments with half operated seeds (*fig. 6*) have shown that a Pfr level above the dark level accelerates the earlier stages of the processes. It can, therefore, be supposed that the increased 'growth potential of the embryo' (BLACK 1969). which is required to overcome the block imposed by the seed-coat, is in fact an acceleration of the first Pfr dependant processes.

If the seed-coat acts primarily as a mechanical barrier, its effect on the Pfr requirement will be rather passive. It can also be supposed, however, that the surrounding layers inhibit the mechanism of the primary action of Pfr more directly. Such an inhibition will certainly also increase the rate of the dark reversion of Pfr to Pr and therefore increase the Pfr requirement still more. It can be assumed, moreover, that the seed-coat thickness interferes in some way with the synthesis of the reaction component of Pfr, which we postulated before (KARSSEN 1970c). The first dependent reactions depend strongly on this factor.

Seeds which do not require the first phase of increased Pfr levels germinate much earlier than red light dependent seeds (compare e.g. *figs. 3* and *6*). It is possible that in the dark germinating seeds the rather slow dark reactions, which prepare the effect of a red irradiation (KARSSEN 1970c) are redundant. The absence of a thick seed-coat can, however, also increase the rate of the dark reactions.

We concluded (see 3.3.) that the Pfr requirement during the last 20 hours of the germination process decreases to below 0.02. It must be noted, however, that it does not imply that the actual Pfr level decreases to the same extent. The dark level of 0.07 presumably remains present. Also in several non-photoblastic seeds of *C. album* the Pfr requirement of the seeds can be lower than the Pfr level being present in the seeds in darkness (KARSSEN 1970b). Such a situation explains why the inhibiting effect of an increase of the Pfr requirement by incubation in mannitol in darkness, diminishes with increasing time between red irradiation and mannitol application (*fig. 11*).

For some time after the splitting of the outer seed-coat layer (the first visible germination phenomenon in *C. album* seeds) the germination mainly consists of cellular expansion. It is unknown whether Pfr prepares for this expansion during the phase of increased Pfr requirement. A different site of action can, therefore, not be excluded.

Another still unsolved problem is why the germination processes do not restart spontaneoulsy when the repression of the inverse dark reversion by prolonged far-red light is stopped (*fig. 9*). BOISARD (1969) suggests that it is both an effect of the deterioration of the inverse dark reversion reaction and of the enzymatic destruction of the small amount of Pfr, which remains during far-red irradiations. The latter point was originally supposed by HARTMANN (1966). NEGBI *et al.* (1968) proposed that an effect on a substrate of Pfr is involved. In view of the present results it can be supposed, in addition, that the Pfr requirement of the processes has been increased again to above the dark level after a long suppression of their activity.

It must be noted, lastly, that the present results do not supply new arguments for our previously reported hypothesis (KARSSEN 1970b), that the last stage of the overall germination is regulated by seedling phytochrome instead of seed phytochrome.

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