THE LIGHT PROMOTED GERMINATION OF THE SEEDS OF CHENOPODIUM ALBUM L. IV. EFFECTS OF RED, FAR-RED AND WHITE LIGHT ON NON-PHOTOBLASTIC SEEDS INCUBATED IN MANNITOL

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SUMMARY

The effects of red, far-red and white fluorescent light on the germination of dark germinating seeds of *Chenopodium album*, incubated in a range of mannitol concentrations, have shown that the far-red absorbing form of phytochrome (Pfr) is present in these seeds. The requirement of the germination processes for Pfr increased, when the osmotic concentration of the incubation medium was increased. It is concluded that this requirement for Pfr depends on the position of a balance between promotive and inhibitory factors. Pfr influences this balance in a positive direction. It was shown that osmotic stress, a thick seed-coat and (RS)-abscisic acid can function as inhibitory factors. In its influence on the last stage of the visible germination phenomena (the protrusion through the inner seed-coat layer) white light resembles in lower osmotic concentrations red light, in higher concentrations it resembles far-red light. The earlier stages (elongation inside the seed) are influenced, in all tested concentrations, similar by red and white light. It is assumed that during the last phase of the germination process "seedling phytochrome" instead of "seed phytochrome" has a regulatory function.

1. INTRODUCTION

The seed germination of a great number of species is influenced by irradiation with white light. The reaction of such photoblastic seeds can be positive (light promotes) or negative (light inhibits). Other seeds do not react upon irradiation with white light. Those seeds are either dormant (no germination in light nor darkness) or non-photoblastic (germination in light as well as in darkness).

In a previous study (KARSSEN 1970) we have shown that the pre-harvest photoperiodical conditions determine whether genetically identical plants of *Chenopodium album* produce either dormant, positive photoblastic or non-photoblastic seeds. The genetical identity of those seeds makes it reasonable to suppose that the phytochrome pigment, which regulates the germination of the positive photoblastic seeds of this species (KARSSEN 1967) is also present in the other seeds.

It seems, however, that phytochrome can not evoke the germination of the dormant seeds and, on the contrary, has no function in the non-photoblastic seeds. In the present study we will investigate these two problems, by means of the seed material obtained from those previous experiments.

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It was demonstrated for several species that non-photoblastic seeds can be rendered photoblastic with a prolonged irradiation with far-red light (HENDRICKS *c.s.* 1959; EVENARI 1965; MANCINELLI *c.s.* 1966; ROLLIN & MAIGNAN 1966). Therefore it is generally accepted that the capacity of these seeds to germinate in darkness is due to the presence of the far-red absorbing form of phytochrome (Pfr) in the dark imbibed seeds, as was originally proposed by TOOLE (1961).

Recently Pfr could indeed be detected by spectrophotometry in dark germinating seeds of *Lactuca sativa* var. "May Queen", *Nemophila insignis* and *Sinapis alba* (BOISARD *c.s.* 1968; BOISARD 1969), *Amaranthus caudatus* var. *viridis* (KENDRICK *c.s.* 1969) and *Cucumis sativus* (SPRUIT & MANCINELLI 1969). Pfr forms respectively 40% (*Lactuca*), 25% (*Amaranthus*) or 75% (*Cucumis*) of the total phytochrome initially present in these seeds.

This "seed phytochrome" differs from the "seedling phytochrome", which is formed in these seeds immediately or some hours after the start of imbibition, in that the Pfr form of the seed phytochrome appears to be stable, whereas this pigment form in seedlings is rapidly destroyed in a dark reaction.

These studies suggest that seed phytochrome is the pigment regulating the germination response. The capacity of this pigment to converse Pr to Pfr in darkness ("inverse dark reversion") explains why the germination of most non-photoblastic seeds can only be inhibited by an irradiation with far-red light over a long period of time.

The incapacity of white light, and therefore most probably Pfr, to evoke in certain seeds of *C. album* a germination response appears to be caused by the thickness of the seed-coat, as was shown previously (KARSSEN 1970). In the present study we will investigate by means of mannitol, in more detail the relation between such a limiting factor and the pigment. The effect of mannitol is usually attributed to an osmotic inhibition of water uptake, preventing the elongation of the cells (HABER & LUIPPOLD 1960).

It was found that mannitol can render dark germinating seeds both positive photoblastic (KAHN 1960; SCHEIBE & LANG 1967) and negative photoblastic (MCDONOUGH 1967). Because the reaction to white light determines whether the photoblastic reaction is called either positive or negative, this light source will be used, together with the more limited wavelength sources red and far-red light.

2. MATERIAL AND METHODS

The seeds were obtained from the experiments described in our previous paper (KARSSEN 1970). Because the quanttieis of seeds, raised in one photoperiodical program, were rather small, a certain phenomenon could not always be investigated with the same lot of seeds. In such a case seeds with a similar degree of dormancy were used.

The storing and germination conditions were described before (KARSSEN 1970). Before incubation in mannitol the seeds were sterilised in a 2% Ca-hypochlorite solution and subsequently washed several times in water. The

petri-dishes, with seeds, filterpaper and the medium, were weighed at the start of the incubation. Every one or two days the dishes were weighed again and brought back to the original weight with water, to assure a rather constant concentration.

The visible germination phenomena can be divided in three stages: I. the outer testa layer is split in the area overlying the radicle (see *fig. 2* in KARSSEN 1968); II. the radicle, still enclosed by the inner seed-coat layer and one endosperm cell layer has extended from within the seed (*fig. 3*, ibid.); III. the radicle has protruded through the inner layers (*fig. 4*, ibid.).

White light was obtained from three white fluorescent tubes (Philips TLF 40 W/33). The light had an intensity of 300 μWatt.cm^{-2} between 400 and 500 nm and of 230 μWatt.cm^{-2} between 600 and 700 nm. All intensities were measured at the level of the seeds. Red light was obtained from one red fluorescent tube (Philips TL 40 W/15) filtered by one layer of 3 mm plexiglass (rot 501, Röhm & Haas, Darmstadt). The intensity was 95 μWatt.cm^{-2}. Far-red light was obtained from five 40 Watt incandescent lamps, filtered by three layers of 3 mm plexiglass, one rot 501 and two blau 627 (R & H) and a 10 cm layer of water. This combination transmitted radiation between 700 nm (1.5%) and about 1000 nm. The intensity between 700 and 800 nm was 50 μWatt.cm^{-2}. We will demonstrate (KARSSEN, in preparation) that this far-red light establishes in the seeds a Pfr/P ratio below 0.02. Blue light was obtained from a blue fluorescent tube (Philips TL 40 W/18) filtered by a 3 mm plexiglass layer blau 0248 (R & H). The intensity was 33 μWatt.cm^{-2}. The green safe light was described before (KARSSEN 1967).

3. RESULTS

A continuous irradiation with far-red light has no significant effect on the germination percentage of the seeds used in this study, when they were incubated in water. In *table 1* are presented some examples. The germination of one of the non-photoblastic lots of seeds is, however, strongly inhibited by far-red light when the seeds are incubated in 0.6 M or 0.7 M mannitol (*table 2*). The seeds seem to be rendered negative photoblastic under these conditions because continuous white and blue light have also an inhibiting effect, an unexpected result for a positive photoblastic species. To permit a good interpretation of these results a broader range of mannitol concentrations was tested, in combination with red, far-red and white light or with darkness.

With respect to the effects of the far-red source, used in the present experiments, it can be seen in *table 2* and *fig. 1* that a continuous irradiation with this source inhibits the germination in certain conditions. Considering the recent litterature (see Introduction), it can be concluded that Pfr is also present in the non-photoblastic seeds of *C. album* imbibed in darkness. The Pfr concentration in darkness must be higher than that established by the far-red source (< 0.02 see Methods).

The natural Pfr content enables 50% of the seeds to germinate in mannitol concentrations up to $\pm$ 0.7 M (*fig. 1*, D curve). In higher concentrations the

Table 1. The influence of continuous far-red light on the germination of several groups of seeds, obtained from previously published experiments (KARSSEN 1970).

In the codes for the cultivation conditions the first letter refers to the photoperiodical conditions during the period before flowerbud formation in the life cycle of the motherplant, the second letter to the conditions between that stage and full flowering, the third letter to the conditions in the last stage till harvest. L: long-days (18 hours); S: short-days (8 hours); R: short days with an interruption of 1 hour red light in the middle of the dark period.

	Cultivation conditions	Percentages germination in continuous		
		White light	Darkness	Far-red light
Exp. 1967A	SSS	100	99	100
	RRR	100	96	97
	LRR	95	65	68
	LRL	71	45	41
Exp. 1968B	RRR	100	98	100
	LSS	100	98	94
	LLL	90	42	36

Table 2. Effect of irradiation with different light sources on the germination of LSS-seeds (Exp. 1967A) incubated in mannitol or water.

Condition	Percentages germination, when incubated in:		
	0.6 M mannitol	0.7 M mannitol	water
Dark	87	68	96
Red	90	70	100
Far-red	7	1	93
White fluorescent	21	4	100
Blue	80	8	95

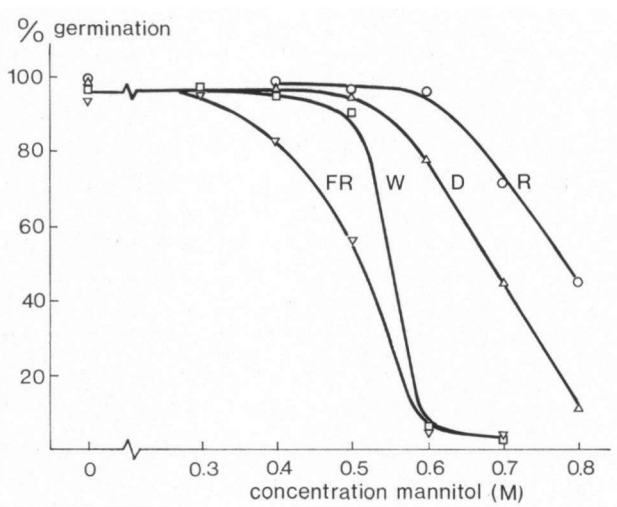


Fig. 1. The effects of an incubation in mannitol of different concentrations on the germination percentages (stage III) of LSS-seeds (Exp. 1968B) (see table 1 for explanation of codes), when the seeds areirr adiated during the whole experiment with red (R), far-red (FR or white (W) light or held in darkness (D). The data are the mean of two experiments with two dishes each.

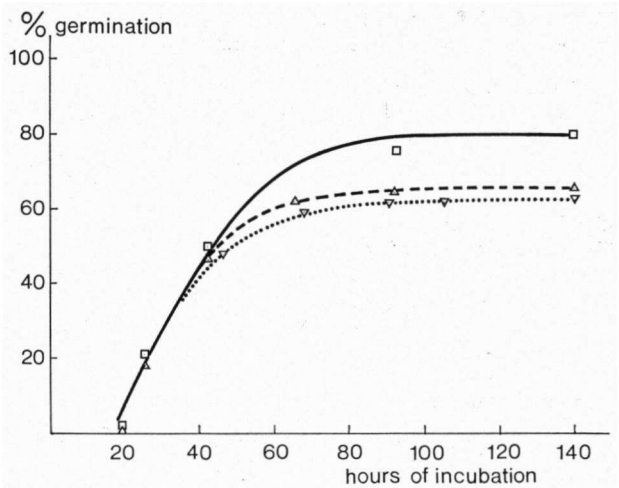


Fig. 2. The time courses of water incubated LSL-seed (Exp. 1968B) irradiated continuously with white (□) or far-red (▽) light or held in darkness (△).

Pfr/P ratio must be increased with red light to 0.81 (BUTLER *c.s.* 1964). In the highest concentrations (above ± 0.8 M) even such a high Pfr level can not evoke a half maximal germination response. A decrease of the Pfr level below the dark level by means of a continuous far-red irradiation prevents this response in mannitol concentrations above ± 0.5 M. From ± 0.5 M to lower concentrations such a far-red treatment can not prevent the 50% response. In water the time course is also unaffected by far-red light (*fig. 2*). In general it is evident from these results that the requirement for Pfr increases with increasing osmotic concentration of the medium.

The ineffectiveness of a far-red irradiation at lower osmotic concentrations means, either that the germination process in such conditions is saturated with Pfr concentrations below the level established with the far-red light source used, or that it is completely independent of Pfr.

The last mentioned explanation should particulary be correct when the Pfr which remains under most far-red sources, is destroyed, as is assumed by HARTMANN (1966). He supported his assumption with the experimental observation that an irradiation with far-red light, or with a combination of a red and a far-red source, which established a Pfr/P ratio of 0.03, has the most effective inhibitory influence on the germination of lettuce seeds (see also BOISARD 1969). Lower Pfr/P levels were less effective. In his view the destruction is saturated with this 0.03 level of Pfr. At lower levels the destruction does not take place and therefore the irradiation is not effective. At the moment that the far-red irradiation can be ended without a germination response in the subsequent dark period (EVENARI 1965; ROLLIN & MAIGNAN 1966; BOISARD 1969), this destruction should have removed the Pfr fraction and, moreover, a great deal of the total phytochrome.

In the present experiments too the far-red irradiation could be ended at a

Table 3. The influence of two transfer treatments, with two groups of seeds. The seeds stayed in the second conditions till the germination percentages remained constant.

a. Continuous incubation in 0.6 M mannitol.
Transfer from far-red light to darkness

	RRR (Exp. 1968B)	LSS (Exp. 1967A)
Continuous darkness	77	83
Transfer to darkness after 1 day	78	–
– 2 days	34	46
– 3 days	15	–
– 4 days	10	5
– 7 days	–	2
– 9 days	–	2
Continuous far-red	0	7

b. Continuous irradiation with far-red light.
Transfer from 0.6 M mannitol to water

	RRR (Exp. 1968B)	LSS (Exp. 1967A)
Continuous water	100	93
Transfer to water after 1 day	100	–
– 2 days	99	87
– 3 days	97	–
– 4 days	89	90
– 7 days	–	84
– 9 days	–	86
Continuous 0.6 M mannitol	0	7

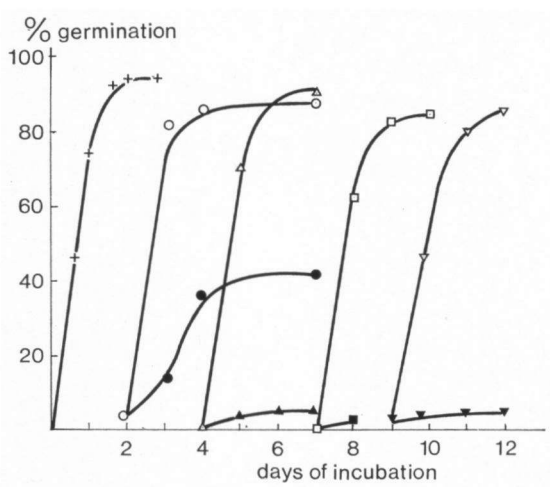
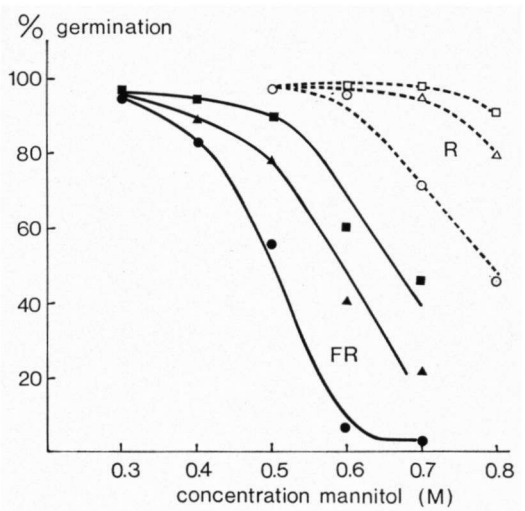
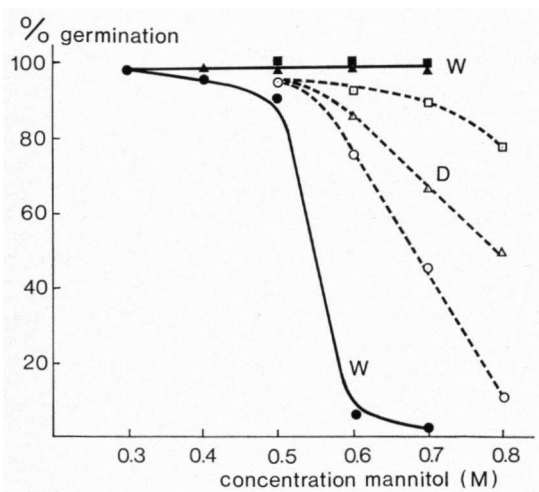


Fig. 3. The time courses of LSS-seeds (Exp. 1967A) which were incubated immediately in water (+) or after 2 days (○), 4 days (△), 7 days (□) or 9 days (▽) of incubation in 0.6 M mannitol. All groups were irradiated continuously with far-red light. The identical closed symbols represent the seeds which were transferred to darkness but stayed in 0.6 M mannitol (see also table 3).

Figs. 4 and 5. As fig. 1, but now represented with the three successive stages of the visible germination phenomena; stage I. (□), stage II (△) and stage III (○).





certain moment, without a restoration of the germination capacity in the subsequent dark period (*table 3a*). The rapid germination response of the seeds when they are transferred after 2 to 9 days from 0.6 M mannitol to water, under a continued far-red irradiation (*fig. 3; table 3b*) should be a strong indication for a Pfr-independency of the processes in water, when the hypothesis of Hartmann is correct.

The observed stability of the seed phytochrome pool, even in continuous far-red, does not support, however, this hypothesis. When Pfr indeed is not destroyed it can not be excluded that the germination processes, which proceed in

continous far-red light, are regulated by a small concentration of Pfr. Then it is, however, not clear why ending the far-red irradiation after some time does not restore the germination capacity. It can be assumed that some unknown reaction during such long incubation periods causes a secondary dormancy. Transfer to water restores, however, the germination capacity immediately, even in continued far-red, and, moreover, at the same rate as before (*fig. 3*).

It is impossible to decide on the moment whether the germination process in continuous far-red is Pfr independent or not. We can only conclude that the requirement for Pfr in very favourable conditions is at least very low.

The complete restoration of the germination capacity, both in percentage (*table 3b*) and in rate (*fig. 3*), after a transfer of the seeds from 0.6 M mannitol to water is a strong argument against a possible damaging effect of a mannitol incubation.

A continuous irradiation with white fluorescent light had in this experiment the same effect as red light up to a concentration of 0.5 M mannitol. In combination with 0.6 M, however, it suddenly acts like far-red light (*fig. 1*). When the effects on the different stages of the visible germination process are compared it appears that the influence of white light is even more complicated (*figs. 4 and 5*). In red and far-red light and in darkness the sensitivity for mannitol increases in a similar way when the elongation of the embryo proceeds. In white light there is, however, a striking difference between the effects on stage I and II – influenced in the same way as in red light – and stage III. The protrusion through the inner seed-coat layer is suddenly prevented at 0.6 M in white light.

The effects of the different irradiations, in combination with a range of mannitol concentrations, on two other lots of seeds, LSL- and LLL-seeds, (*figs. 6*

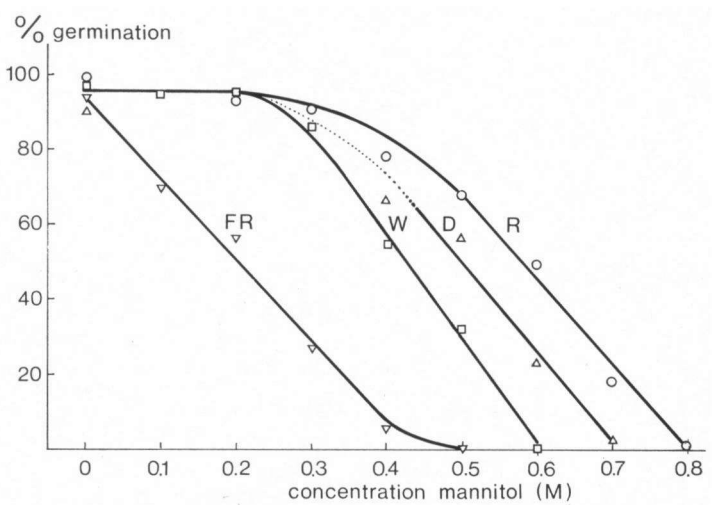


Fig. 6. As *fig. 1*, but for LSL-seeds (Exp. 1968B).

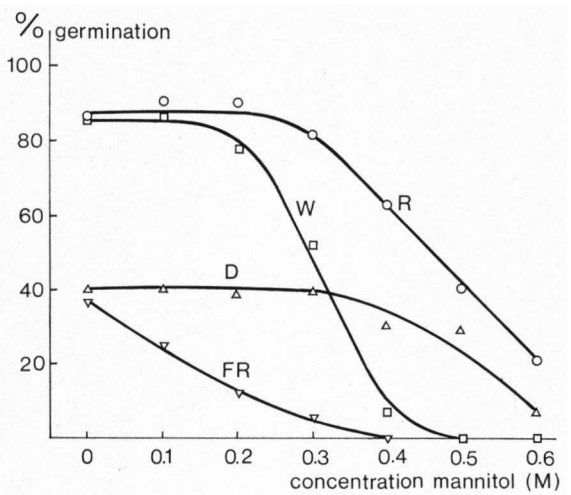


Fig. 7. As *fig. 1*, but for LLL-seeds (Exp. 1968B).

and 7) show a qualitative resemblence to the results presented in *fig. 1* for the LSS-seeds. There are, however, some quantitive differences. This can be seen from the mannitol concentrations that allow 50% germination when the seeds are irradiated with red light: 0.78 M (LSS), 0.59 M (LSL) and 0.49 M (LLL). It is important to notice that the sensitivity to mannitol increases in the same sequence as the degree of dormancy of these seeds (LSS – LSL – LLL) (KARSSEN 1970). It was concluded in that paper that after some months of dry storage the dormancy depends in particular on the thickness of the seed-coat. Seeds with a thick seed-coat (LLL) show a high mannitol sensitivity, whereas seeds with a thin seed-coat (LSS) have a low sensitivity to mannitol. Seed-coat thickness and osmotic concentration appear to act at least additive.

The rather similar differences in the mannitol concentrations that allow 50% germination in respectivily darkness and red light in the three groups of seeds (0.69 M and 0.78 M for the LSS-seeds, 0.51 M and 0.59 M for the LSL- seeds, 0.51 M and 0.60 M for the dark germinating seeds of LLL) present an indication that the Pfr levels in darkness of these seeds are rather similar. Therefore this factor can not be responsible for the differences in degree of dormancy in darkness, as obtained in our previous study (KARSSEN 1970). The conclusion in that paper, that mainly morphological factors are responsible for different germination capacity of the seeds is thus supported by these results.

The sensitivity for another germination inhibitor (RS)-abscisic acid (ABA) follows also the sequence LSS-LSL-LLL (*fig. 8*). The stronger inhibitory effect of this growth regulator in darkness than in red light completely supports our earlier results (KARSSEN 1968). And so does the observation that only stage III is inhibited by ABA.

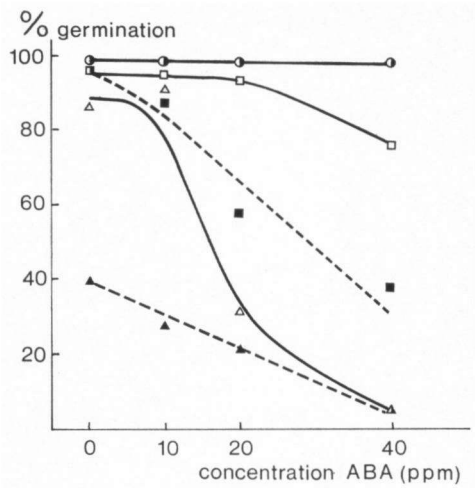


Fig. 8. The germination percentages (stage III) of LSS-seeds (○), LSL-seeds (□) and LLL-seeds (△) (Exp. 1968B) incubated in (RS)-abscisic acid in continuous red light (open symbols) or darkness (closed symbols).

4. DISCUSSION

4.1. Function of Pfr in germination

Photoblastic seeds, both positive and negative, have mostly been used as objects for the study of the function of phytochrome in seeds. The present results have shown once more that also in non-photoblastic seeds Pfr can be present. In several seeds this Pfr fraction regulates the dark germination in water (see Introduction for references). It is a striking feature that such seeds are not present among the seeds used for the present experiments (*table 1*). It is possible that this characteristic had disappeared during the dry storage period. Most experiments were done 5 to 6 months after harvest. The present results showed that in these seeds the germination processes are either completely independent of Pfr or saturated with very low Pfr levels.

It is evident that phytochrome regulated germination is not restricted to the naturally photoblastic seeds. It can be assumed that the phytochrome pigment is present in every seed (McDONOUGH 1967). The demonstration of so many different effects of the pigment, in every part of the plant (MOHR 1966) and in a great number of species, even suggests that it belongs to the normal biochemical components of the plant cell. Its active regulation of the metabolic processes in seeds seems to depend, however, on several non-photochemical factors.

The demonstrated relation between the requirement for a certain Pfr level and the osmotic concentration of the incubation medium (*figs. 1, 6* and *7*) is a good example of this hypothesis. It was demonstrated that the ability to germinate either in darkness, with the natural Pfr level, or in red light, with a maximal Pfr level, can also depend on the concentration of ABA in the medium (*fig. 8*) and on the morphological characteristics of the seeds (*fig. 4* in KARSSEN 1970). The comparison of the three groups of seeds, used in the present experiments revealed that mannitol concentration and seed-coat thickness act at least ad-

ditive. Also the sensitivity for ABA increases with increasing seed-coat thickness (*fig. 8*).

Requirement for red light could also be induced with several other factors, like for instance coumarin, 2,4-D, x-rays and high oxygen tension. Factors such as gibberellins, kinetin, thiourea, nitrates and high CO_2 tension promote the dark germination of several photoblastic seeds (see EVENARI 1965 for references). It is unlikely that all these factors act directly on the phytochrome system.

It can be assumed therefore that the germination capacity of seeds depends on a balance between promotive and inhibitory factors. This balance can be influenced in a positive direction by Pfr. When the overall situation is already promotive, the seeds are Pfr independent or saturated with very low Pfr levels. Only when the balance is just subcritical to the Pfr level in darkness, red light has a positive effect. When the balance is too negative, the seeds are dormant even in red light. This restricted effect of light was clearly shown in *fig. 3* of KARSSEN (1970). An irradiation with white light caused only a small positive shift in this balance, which was determined by pre-harvest conditions.

4.2. White light effects

It was concluded from *table 2* that an incubation in 0.6 M and 0.7 M mannitol brought about a negative photoblastic response of the seeds. The more detailed results (*figs. 1, 6* and *7*) have shown that the sign of the photoblastic response depends, however, strongly on the concentration of the incubation medium. It can be seen in *fig. 1* that the LSS-seeds can be called non-photoblastic (no light effects) in concentrations below 0.3 M; apparent non-photoblastic (far-red light inhibits) between 0.3 and 0.5 M; and negative photoblastic (white light inhibits) at 0.6 M. The promotive effect of red light in higher concentrations is a typical positive photoblastic response.

These results support the relative value of the division of the seeds in these categories (EVENARI 1965). This was also demonstrated by the influence of the temperature on the sign of the photoblastic reaction (KOLLER & NEGBI 1959; KENDRICK & FRANKLAND 1969). It was also observed that in several seeds a short irradiation with white light gave a positive response like red light, whereas a prolonged irradiation inhibited, like far-red light (ISIKAWA 1957; KOLLER & NEGBI 1959; NEGBI & KOLLER 1964).

The present results show a similar contradiction. White light resembles red light in its influence on stage I and II of the visible germination phenomena, whereas it resembles in a great deal far-red light in its effect on stage III. It is important to remember that the seeds have reached stage III when they have protruded through the inner seed-coat layer and therefore have changed from an elongation of the embryo within the seed, to a real seedling growth outside the seed.

The similarity between the effects of red and white light on the stages within the seed fully agrees with the properties of a stable seed phytochrome. Both light sources can establish a high Pfr/P level. Red light gives 0.81, while

KENDRICK & FRANKLAND (1969) found for white fluorescent light a ratio of 0.75

It will be discussed whether the different effects of white and red light on stage III can be explained with the hypothesis that in that phase the seedling phytochrome has taken over the regulatory function from the seed phytochrome. As was mentioned in the Introduction this second pool of phytochrome is formed either immediately after the start of the imbibition, some hours before or during the appearance of the visible germination phenomena.

The destruction rate of seedling phytochrome is directly related to the proportion of phytochrome in the Pfr form (KENDRICK & FRANKLAND 1968). Therefore only prolonged or continuous irradiations with light sources that establish low Pfr/P levels can assure the presence of a certain level of the Pfr form of seedling phytochrome for a required long time. Far-red and, to a smaller degree, blue light cause thus the strongest effects, whereas red light has no effect or only a small one.

Seed phytochrome has, as was mentioned before, always a promotive effect on the growth processes within the seed. It was observed, however, in lettuce and other species that seedling phytochrome inhibits the first developmental stage after the visible germination phenomena (the lengthening of the hypocotyl) (HARTMANN 1966).

When the presumed shift from seed phytochrome to seedling phytochrome is at the same time a shift from a positive to a negative response, it could not be detected during the continuous irradiations with red and far-red light in the present experiments. Red light will cause in both phases the strongest positive biological response, far-red light the strongest negative biological response.

The two pigment forms differ, however, also in their dependency on the light intensity. The effects of the different light sources on the germination process are intensity independent. Also the inhibitory effect of far-red light seems to depend only on the duration of the irradiation. It was shown, for instance, in tomato seeds that a continuous irradiation with far-red light had a similar effect as an intermittent irradiation for only 3.3% of the time (MANCINELLI *c.s.* 1966; YANIV *c.s.* 1967). Biological responses regulated by seedling phytochrome are, however, strongly dependent on the intensity of far-red and blue light (WAGNER & MOHR 1966; HARTMANN 1966; KENDRICK & FRANKLAND 1969). When prolonged irradiations with red light has an effect at all on such a response that effect is independent of the intensity. The coincidence of the blue and far-red curves in the intensity-response graphs of WAGNER & MOHR (1966) and HARTMANN (1966) suggests that the Pfr/P ratio, established by the light source, is not important in this intensity effect.

Because the white light, used in the present experiments, had a rather high intensity, especially in the blue wavelenghts band, it can indeed be expected that it has a promotive effect before the shift (stages I and II) and an inhibitory effect after it (stage III). The blue light source, which could be used in the present study in only one experiment (*table 2*), also causes an abrupt inhibition between 0.6 and 0.7 M mannitol.

This hypothesis seems to be supported by the results of ROLLIN & MAIGNAN

(1967). Their interpretation of the light effects on the germination of the negative photoblastic seeds of *Phacelia tanacetifolia* and *Nemophila insignis* corresponds, in our opinion, completely with the properties of seedling phytochrome and not with those of seed phytochrome. Especially the smaller inhibitory effects of a far-red light irradiation, which is preceded by a red irradiation (see also MANCINELLI *c.s.* 1967) agrees with the action of an unstable pigment (MOHR *c.s.* 1965; GRILL & VINCE 1969). It is important to notice that in the seeds of *Nemophila* also seed phytochrome is present (BOISARD 1969). The delay of the germination rate in continuous red light, found by KENDRICK & FRANKLAND (1969) in *Amaranthus caudatus* seeds fits in the scope of our hypothesis.

An interesting consequence of our hypothesis is that the strongly different effects of white light on the germination of seeds may depend on the relative importance of the two phytochrome forms. It depends moreover on the balance between promotive and inhibitory factors within the seeds.

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