THE LIGHT PROMOTED GERMINATION OF THE SEEDS OF CHENOPODIUM ALBUM L. V. DARK REACTIONS REGULATING QUANTITY AND RATE OF THE RESPONSE TO RED LIGHT

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SUMMARY

In *Chenopodium album* seeds the influence of the dark incubation time before a red irradiation on the subsequent germination processes was studied.

During dark incubation at least two preparative reactions take place. The first one, a rehydratation of the phytochrome pigment, is both at 23 °C and 4 °C a function of the water uptake of the seeds. It regulates the rate of increase in reactivity of the seeds to red light, as measured by the final germination percentage. In the second one a reaction component for the far-red absorbing form of phytochrome (Pfr) is synthesized. This synthesis does not occur at 4 °C. The amount of this component synthesized before onset of a red irradiation, determines the reaction rate of Pfr and consequently the escape from the antagonistic effect of a far-red irradiation, which in turn determines the moment of visible germination. The processes between the moment of escape and the moment of the protrusion of the rootlet through the inner seed-coat layer are not influenced.

The lower final germination percentage, after a 4 °C pre-treatment, as compared with incubation at 23 °C, is supposed to be caused by the dark conversion of Pfr to Pr in the absence, at the lower temperature, of the product of the second reaction.

1. INTRODUCTION

It has been reported in the first paper of this series (KARSSEN 1967) that the reactivity of the seeds of *Chenopodium album* towards a saturating irradiation with red light – measurable by the final germination percentages – increased during the first 24 hours of incubation in darkness. After 48 hours it decreased again. A determination of the time courses of the visible germination phenomena showed that the irradiation moment also influenced the average length of the post-irradiation period, i.e. the period of time between red light and the half time of visible germination. The incubation time before red light determined, therefore, both the final result and the rate of the processed initiated by the irradiation. In the meantime the second effect was also found by NEGBI *c.s.* (1968) in lettuce seeds.

In the present study a comparison was made between the influence of a temperature of either 4 °C or 23 °C during the pre-irradiation period, in order to analyse the dark processes, regulating these two effects. Moreover it was tried to determine whether the rate of all partial processes during the post-irradiation period is influenced or only of a certain part of them.

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2. MATERIAL AND METHODS

The seeds (code number 147) used for the present experiments were harvested in 1966 from a group of plants on a waste lot near Utrecht. The storing conditions and the germination methods employed have been described before (KARSSEN 1967, 1968).

Red light was obtained from two red fluorescent tubes (Philips, 40 W/15), filtered by one layer of 3 mm plexiglass (rot 501, Röhm & Haas, Darmstadt). The intensity was 200 μWatt.cm^{-2} at the level of the seeds. The far-red light source (intensity 50 μWatt.cm^{-2} between 700 and 800 nm) has been described before (KARSSEN 1970a), just like the green light source used for the determination of the time course of dark incubated seeds (KARSSEN 1967). The red irradiations had in all experiments a duration of 15 min (18.10^4 μWattsec.cm^{-2}), the far-red irradiation of 30 min (9.10^4 μWattsec.cm^{-2}).

When the seeds were incubated at 4 °C the dishes, with filterpaper and deionized water, were pre-incubated at that temperature for at least 8 hours, before the seeds were sown.

For the determination of the rate of water uptake, the seeds were weighed in dry condition and reweighed after a certain time of dark incubation. Before the second weighing the seeds were surface dried by pressing them between two layers of dry filterpaper, during half a minute.

The time course of the visible germination phenomena was determined in all experiments. The experiment was ended when the germination percentages remained constant for about two days.

3. RESULTS

In order to analyse the processes during the pre-irradiation period the seeds were placed in darkness immediately after the sowing in water, either at the normal incubation temperature of 23 °C or at 4 °C. After a variable time the seeds, which had been incubated at 4 °C, were transferred to 23 °C. Immediately afterwards they were irradiated for 15 min with red light together with the 23 °C controls. All seeds were then incubated at 23 °C till the germination percentages remained constant. The final germination percentages, which were attained after these treatments, are shown in *fig. 1*.

There is a good qualitative resemblance between the 23 °C curve and the results reported before (KARSSEN 1967). The maximum response of the seeds used in that study was, however, 90%, whereas in the present investigations a maximum of 50% was attained. In the next paper (KARSSEN 1970b) it will be shown that this seed material can reach the 90% level only after repeated or continued red irradiation.

It can be seen in *fig. 1* that the reactivity towards a red irradiation for 15 min increases also during incubation at 4 °C. The rate is, however, slower than at 23 °C and the maximal percentages are lower.

IKUMA & THIMANN (1964), who obtained rather similar results with lettuce seeds, found a correlation between the increase in reactivity to red light and

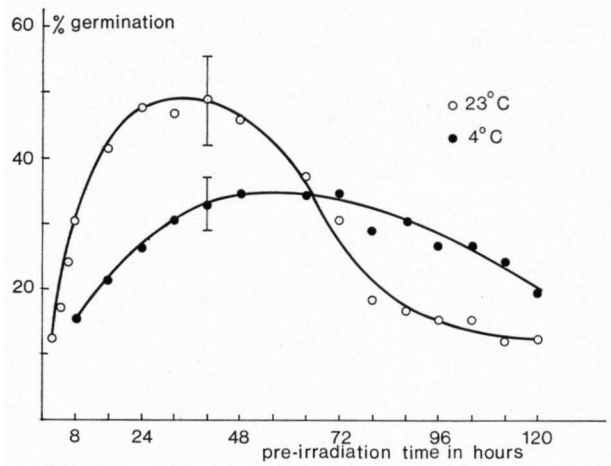

Fig. 1. The effect of two temperatures on the reactivity of the seeds to a single red irradiation (15 min) after different periods of dark incubation. The seeds were incubated at 4 °C only before the irradiation. During and after irradiation the temperature was always 23 °C. The points are the mean of two to twenty experiments with two dishes each. The vertical bars represent the standard deviations of the mean.

the rate of the increase in fresh weight at different temperatures. A comparison between *figs. 1* and *2* reveals that the seeds of *C. album* at the moment of maximal reactivity (after 24 hours at 23 °C and 48 hours at 4 °C, *fig. 1*) have nearly the same fresh weight increase (22.5%, *fig. 2*). Ikuma & Thimann found also that the seeds have not to imbibe to the maximum to attain maximum response. The quotient between the increase in fresh weight per hour in the more or less linear part of the curves at 23 °C and at 4 °C is 1.36. This value can be compared with the quotient between either the actual increases in reactivity to red light at both temperatures or the relative ones. These amount to 2.04 and 1.38, respectively. To obtain the relative increase the maximum percentage at both temperatures was set at 100 and the percentage in darkness at 0, the intermediate percentages were adjusted accordingly. It can be concluded from these values that the rate of increase in reactivity to red light is a function of the rate of water uptake. The difference in the maximum percentages at both temperatures is caused, however, by another temperature sensitive factor.

The experimentally obtained relation between the length of the pre-irradiation time (either at 23 °C or at 4 °C) and the length of the post-irradiation time (always at 23 °C) is represented in a diagram (*fig. 3*). It can be seen that the post-irradiation time has a constant length of 57 to 58 hours when red light is given after 40 to 72 hours of incubation in darkness at 23 °C. When the pre-irradiation time at 23 °C is shorter than 40 hours, the post-irradiation time is prolonged to 75 hours.

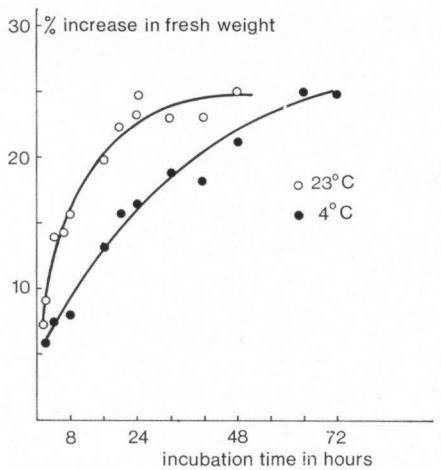

Fig. 2. The effects of two temperatures on the water uptake of the seeds in darkness. The points are the mean of three experiments with two dishes each.

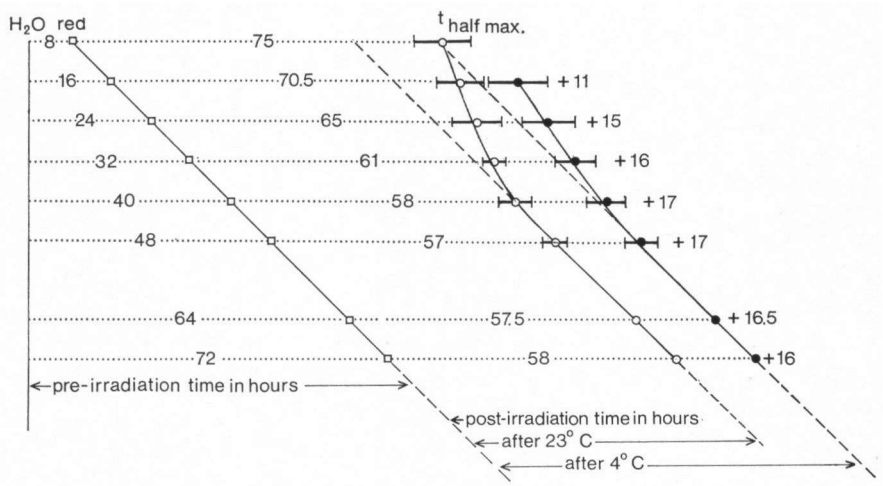

Fig. 3. A diagrammatic representation of the influence of the length of the pre-irradiation time (at 23 °C or 4 °C) on the length of the post-irradiation time (at 23 °C). H_2O = start of incubation in darkness; red = moment of irradiation with red light (15 min); $t_{half\ max}$ = germination half time. The same number of experiments as in fig. 1. The difference between the lengths of the post-irradiation time after 23 °C and 4 °C (see the number to the right) is not significant after a pre-irradiation time of 16 hours, significant ($p = 0.05$) after 24 hours, highly significant ($p < 0.01$) after later irradiation moments (according to Students t test).

It appears that a fast reaction after the irradiation requires a factor which is not yet present after 8 hours at 23 °C, but has been formed to a maximum after 40 hours. Although the post-irradiation time is longer after an irradiation at the 8th hour than after one at the 40th hour, the total germination time (from the start of the incubation to $t_{half\ max.}$) increases nevertheless in the opposite direction. It can be concluded, therefore, that it saves time when red light is given during the formation or activation of the factor.

An incubation at 4 °C before red light lengthens the post-irradiation time with 15 tot 17 hours (*fig. 3*). It can be concluded therefore that the factor, which regulates the rate of the processes after red light, is not formed during an incubation at 4 °C. Its formation starts only after the seeds are transferred to 23 °C.

It was tried to determine whether the rate of all the post-irradiation processes was influenced or only a certain part of them. The first phenomenon after the red irradiation that can be detected in a simple way is the escape from the antagonistic effect of a short far-red irradiation. It can be seen in *fig. 4* that this reaction starts very rapidly after a pre-irradiation time of 48 hours at 23 °C. After earlier irradiations the reaction starts at a slower rate. The rate of the reaction in the linear part of the curves is, however, identical after all irradiation moments tested. An incubation at 4 °C before red light influences also the escape reaction (*fig. 5*). The reaction has a lag phase of about 10 hours when red light is given after 40 hours at 4 °C, in contrast to the 23 °C control.

The escape curves were characterized by the half time of the process, i.e.

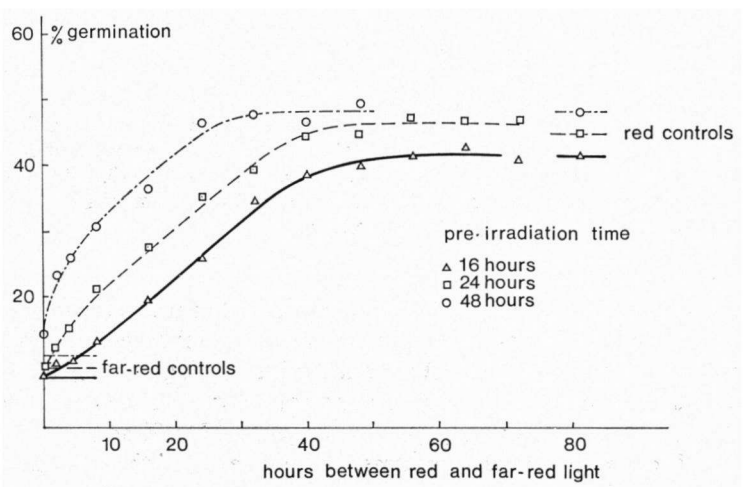

Fig. 4. The influence of the length of the pre-irradiation time (at 23 °C) on the time course of the escape from the antagonistic effect of a short far-red irradiation (30 min) after red light. Means of three experiments with two dishes per treatment.

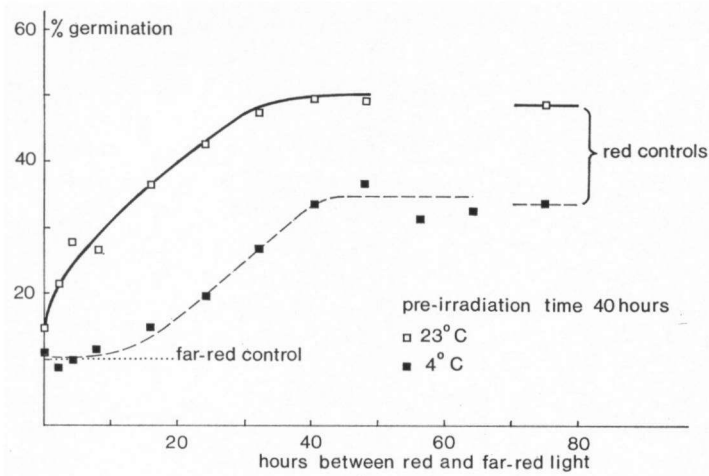

Fig. 5. The effect of the temperature during the pre-irradiation time on the time course of the escape reaction.

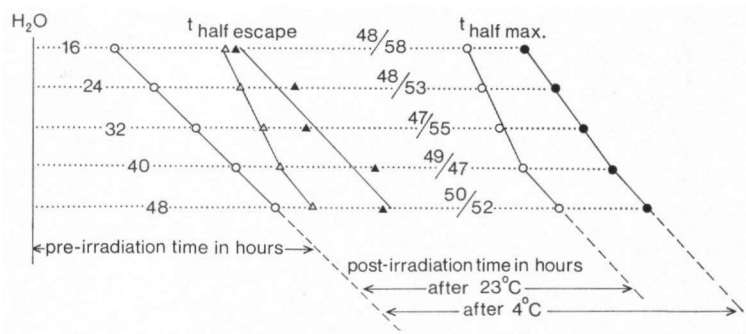

Fig. 6. A diagrammatic representation of the influence of the length of the pre-irradiation period (at 23 °C or 4 °C) on the escape half time ($t_{half\ escape}$) and the germination half time ($t_{half\ max.}$). The row of numbers in the middle indicates the period of time between these two moments, either after a 23 °C pre-treatment (first number open symbols) or after a 4 °C pre-treatment (second number, closed symbols).

the moment whereupon half of the difference between the percentages of the red and the far-red controls could no more be inhibited with far-red light. These escape half times ($t_{half\ escape}$) were fitted into a diagram (*fig. 6*), together with the data from *fig. 3*. At a constant temperature of 23 °C the period between $t_{half\ escape}$ and $t_{half\ max.}$ has a nearly constant length of 47 to 50 hours, irrespective of the length of the pre-irradiation time. The influence of the irradiation moment on the post-irradiation time can thus be reduced completely to an influence on the escape reaction. The lengthening of the post-irradiation time, due to a 4 °C pre-treatment, can be attributed at least after irradiations on

the 40th and 48th hour, to the delay of the escape reaction, because the period between $t_{half\ escape}$ and $t_{half\ max.}$ has nearly the same length after both temperature pre-treatments. The differences at earlier irradiation moments are not significant.

4. DISCUSSION

It can be concluded from the present results that at least two preparative dark reactions regulate the influence of the incubation time on quantity and rate of the response to red light.

One reaction regulates the rate of the increase of the final germination percentage (*fig. 1*). It was concluded that the reaction is a function of the water uptake of the seeds. KENDRICK *c.s.* (1969) found that the kinetics of the appearance of phytochrome spectrophotometrically detectable in *Amaranthus caudatus* seeds are very similar to those of the water uptake. At 0 °C both processes are slowed down to nearly the same degree. They concluded that the increase in detectable phytochrome may either be of a purely optical nature, such as changes in light scatter, or it may reflect the gradual rehydration of an initially photoinactive form of the pigment, present in the dry seeds (see also TOBIN & BRIGGS 1969). In cucumber seeds (SPRUIT & MANCINELLI 1969) "seed phytochrome" could already be detected in dry seeds. It was unaffected by imbibition. The second pool of the pigment (seedling phytochrome) appeared only after the start of the imbibition. Seedling phytochrome appeared in *Amaranthus* seeds after about 10 hours of incubation. That this second phase of phytochrome increase is most likely a synthesis, involving metabolic processes, is indicated by the fact that it does not occur when the *Amaranthus* seeds are imbibed at 0 °C.

All the seeds, in which phytochrome could be detected till now, germinated readily in darkness. Consequently an increase in reactivity to a red irradiation, as reported for *C. album* (*fig. 1*), could not be observed. The influence of the temperature on the rate of this increase in the present results could be completely attributed to an effect on the water uptake. This agreement with the kinetics of seed phytochrome appearance in other seeds makes it reasonable to conclude that the increase of the final germination percentages depends on an increase in the photoreactivity of seed phytochrome, being already present in the seeds, which in turn is a function of an increasing rehydratation of the seeds.

The effects of a variation in the pre-irradiation time either at 23 °C or 4 °C, on the length of the post-irradiation time (*figs. 3* to *6*) provide arguments for the presence of a second preparative dark reaction. The constant lengthening of the period after red light, due to a 4 °C pre-treatment, indicates in the first place that the second reaction is completely inhibited at 4 °C, whereas the first one is only delayed. It is shown in *fig. 3* that a pre-irradiation time of 64 and 72 hours at 4 °C still causes the lengthening of the post-irradiation period, although the fresh weight is identical at these moments at both temperatures (*fig. 2*). It can be concluded, therefore, that the effect of 4 °C on the second

reaction, in contrast to the first one, is not due to an influence on the water uptake. The second reaction appears to be inhibited itself.

Regarding the time course of both reactions it has been shown before (KARSSEN 1967) and could be confirmed in the present study, that whereas the maximal germination percentage is reached at 23 °C after 24 hours (*fig. 1*), the post-irradiation time has its minimum length only after 40 hours (*fig. 3*). The identical length of the post-irradiation time after a pre-irradiation time of either 8 hours at 23 °C or 40 hours at 4 °C (*fig. 3*) indicates that at 23 °C the second reaction does not start immediately after the start of the incubation, in contrast to the phytochrome reactivation. The same conclusion can be derived from the similar escape-half-times after a pre-irradiation time of 16 hours at either 4 °C or 23 °C (*fig. 6*). In general it can be concluded, therefore, that the two reactions have a different time course.

It must be noted that a certain indirect effect of a 4 °C treatment on the length of the post-irradiation period via the water uptake can not be excluded. Most obviously the start of the second reaction is triggered by a certain degree of water uptake. A reduced fresh weight increase at 4 °C will therefore certainly also influence the second reaction. Such an effect can presumably explain the gradual decrease of the post-irradiation times after pre-irradiation times at 4 °C from 16 hours up to 40 hours (*fig. 3*). Although the second reaction always starts only after the transfer to 23 °C, the lower fresh weight increase at 4 °C can cause an extra delay during the first 40 hours.

In general it can be concluded that a second reaction, differing from the phytochrome reactivation, proceeds in the seeds. Because the lengthening of the post-irradiation time could be located completely in a delay of the start of the escape reaction (*figs. 4* and 5), it can be concluded that the second reaction has a close relation to the primary effects of Pfr. Although the escape phenomenon does not elucidate the nature of the primary reactions, it does indicate when the high level of Pfr, established by red light, and being essential for the induction of the germination response, is not required anymore. When far-red light can still prevent the germination response in all seeds, as is the case after a pre-irradiation period at 4 °C (*fig. 5*), Pfr has most obviously not caused an induction in any seed. Because we concluded just now that the photoreactivity of phytochrome has not a regulating function in these time course effects, it can be assumed that another essential component for the primary induction of the germination processes has this function, as was supposed also by NEGBI *et al.* (1968). Whether the component acts either as the substrate of Pfr or in another function can not be decided at present.

It can be assumed that the delay of the immediate action of Pfr enables a certain reversion of Pfr to Pr in darkness. Consequently the concentration of Pfr will be lower at the moment when the concentration of the other reaction component is sufficiently increased to allow a reaction. In this way a possible explanation can be given for the difference between the maxima of the germination response after a pre-irradiation time at 4 °C and 23 °C (*fig. 1*). The smaller response at 4 °C is thus influenced both by the slower rehydration of

phytochrome and by the prevention of the second reaction.

It must be noted, however, that the dark reversion of Pfr to Pr was detected spectrophotometrically in only a small number of studies (see HILLMANN 1967 for references). During the recent estimations of phytochrome in seeds it was attempted to detect the reaction by SPRUIT & MANCINELLI (1969) in cucumber seeds. The simultaneous presence of seedling phytochrome made it, however, quite difficult to establish whether the decrease in Pfr, which was observed after a saturating red irradiation, was due to the dark reversion of Pfr to Pr or to a destruction of Pfr. IKUMA & THIMANN (1964) found in lettuce seeds that anaerobic conditions after the red irradiation stopped the escape reaction. When the aerobic conditions were restored the escape reaction proceeded normally again, but the final germination percentage was decreased. They concluded that during the anaerobiosis Pfr was converted back to Pr. SPRUIT (1967) found that in pea leaves and stem sections such conditions prevent the destruction of Pfr, whereas they did indeed not inhibit the dark reversion. In the next paper of this series (KARSSEN 1970b) more arguments for the presence of the dark reversion reaction in *C. album* seeds will be presented.

Lastly it can be concluded from the present results that also the decrease of the reactivity to red light (secondary photodormancy), which starts after 48 hours of dark incubation at 23 °C and after 72 hours at 4 °C (*fig. 1*), is not correlated with the length of the post-irradiation time, which remains constant for a longer period. In one single experiment it could be observed that the second period still had the constant length of about 58 hours when the irradiation took place between 72 and 96 hours. The remark in our previous report (KARSSEN 1967) that the length of the post-irradiation period increases again after irradiations at the 59th hour and later seems to be unfounded.

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