FURTHER EVIDENCE FOR THE EXISTENCE OF TWO PHYTOCHROME SYSTEMS FROM TWO DISTINCT EFFECTS OF FAR-RED LIGHT ON LETTUCE SEED GERMINATION

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SUMMARY

Thermo-dormant lettuce seeds can be induced to germinate by small doses of far-red light. The dose-effect curve appears to be an optimum curve. After having been irradiated with a supra-optimal inhibiting dose of far-red the seeds can be reinduced by red light, the threshold red dose for induction being now 10^4 times as large as it is without the far-red preirradiation. It is concluded that the inductive effect of far-red light is mediated by an uncommon form of phytochrome which is considered to be also present in etiolated *Avena* seedlings and *Sinapis* seedlings, and in cells of *Mougeotia*.

1. INTRODUCTION

Seeds of lettuce of the cultivar Noran are not photoblastic. Light requirement can be induced by incubating seeds under far-red light or at temperatures above 30 °C (far-red dormant or thermo-dormant seeds respectively). After the far-red pretreatment germination can be induced by red doses of 10^2 μW sec cm^{-2} or more, after the heat pretreatment by red doses of 10^{-2} μW sec cm^{-2} or more. This paper reports on the effect of short far-red irradiations on the extremely light-sensitive thermo-dormant seeds.

2. MATERIAL AND METHODS

The general methods have been described in BLAAUW-JANSEN & BLAAUW (1975). Narrow spectral bands were isolated by means of interference filters ("DEPAL", Schott u. Gen., Mainz). The intensity was reduced, if necessary, by introducing NG (Type neutral) filters (Schott u. Gen.). In some experiments the interference filters were replaced by a perspex red filter combined with an infra-red mirror + a Calflex filter (Balzer, Liechtenstein) or by 1 red and 2 blue perspex filters combined with an infra-red mirror in order to produce red or far-red light of a higher flux density. The red perspex (no 501) and the blue perspex (no 627) were obtained from Röhm und Haas, Darmstadt.

3. RESULTS

The thermo-dormant 'Noran' seeds (imbided at 37°C during 72 hours) can be induced to germinate by short far-red irradiations as well as by red irradiation. Surprisingly the dose-response relation for the far-red induction is an optimum curve. (*fig. 1, curve III*), whereas the dose-response relation for red induction has the shape of a saturation curve (*fig. 1, curve II*). This is true for high red light intensities in short irradiation times (*fig. 1, curve I*) as well as for low intensities being sufficient to produce the maximum germination percentage in the same irradiation time as is done by far-red light (*fig. 1, curve II*).

As these seeds show a response to very small quantities of red light it can be understood that also the small amount of P_{fr} produced by far-red light is sufficient to promote germination. However, the reduction of the germination percentage at larger far-red doses cannot be due to the amount of P_{fr} getting supraoptimal, since at large red doses (producing large amounts of P_{fr}) no reduction of the germination percentage can be observed.

The initial photoproduct in the photoconversion $P_r \rightarrow P_{fr}$ is an intermediate that is transformed to P_{fr} in a series of dark reactions (KENDRICK & SPRUIT 1973). If irradiation with far-red light is stopped before P_{fr} is formed from the intermediates there is no possibility of reconversion to P_r by far-red. It might be conceivable that in that case a higher percentage of P_{fr} could be reached than the stationary 2 to 3 %. In fact the phototransformation of P_r to P_{fr} is reported not to be completed within 1 second (at 0.5 °C), possibly it is in 5 seconds, beyond any doubt only after 20 seconds (LINSCHITZ et al. 1966). If this is correct a higher percentage of P_{fr} should be reached by giving a short irradiation of a high inten-
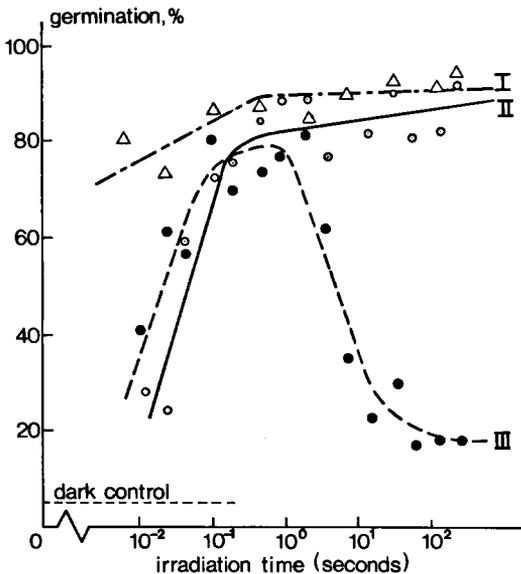

Fig. 1. Dose-response curves for the induction of germination by irradiation
I. 660 nm, I $= 10^3$ μW cm^{-2}
II. 660 nm, I $= 3$ μW cm^{-2}
III. 735 nm, I $= 2.10^2$ μW cm^{-2}

sity than by irradiating at lower intensity during a longer period: the reciprocity law should not be valid. The results of the present experiments do not fit in this hypothesis since it is seen from *fig. 2 A* that a low light intensity is more profitable than a high one, which is in contradiction with the above mentioned expectation. We intend, however, to investigate this provisional conclusion by experiments with higher far-red intensities during shorter irradiation times.

'Noran' seeds treated with red light can be prevented to germinate by subsequent irradiation with far-red light. The far-red doses required for this antagonizing effect are of the order of magnitude of the far-red doses which appeared supraoptimal in the experiments of *fig. 2 B*. This suggests that the descending part of the optimum curve represents the usual inhibiting affect of far-red light on red-induced germination.

The shape of the optimum curve depends on the wavelength for which it is determined. *Fig. 3* shows that for shorter wavelengths the optimum curve is flatter than for longer wavelengths, obviously the ascending part of the optimum curve represents a system with an action spectrum different from that of the descending part. Together the two systems simulate the shape of an optimum curve.

Seeds that received a supraoptimal inhibiting quantity of far-red light (ca 10^4 μW sec cm^{-2}) can be reinduced to germination by red irradiation. The dose of red light required for this reinduction is 10^4 as large as the quantity required for the germination of non far-red irradiated controls (*fig. 4*). So this small dose of far-red light has reduced the sensitivity for red light with a factor 10^4. In a
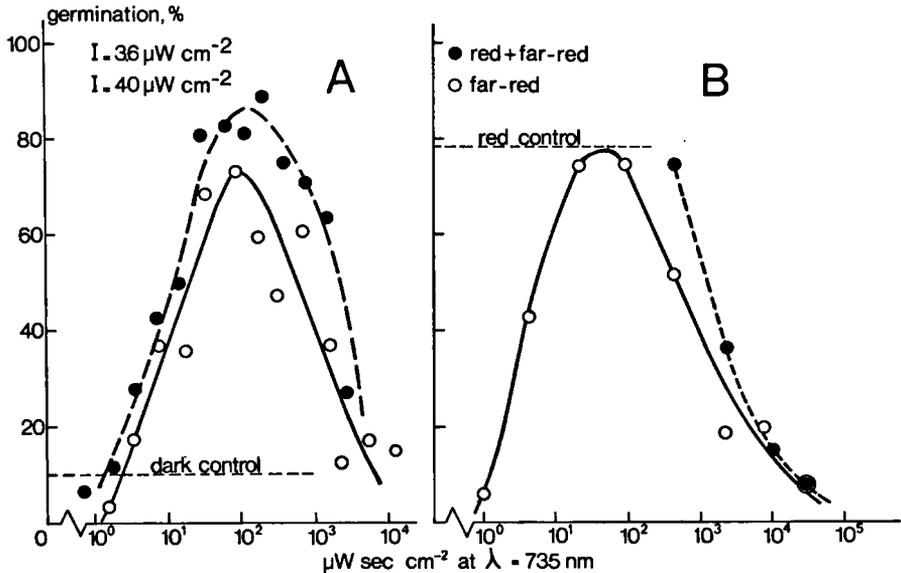

Fig. 2. A. Dose-response curves for induction by far-red irradiation at two intensities.
B. The inhibiting effect of far-red light on red-induced germination in comparison with the effect of far-red light alone.
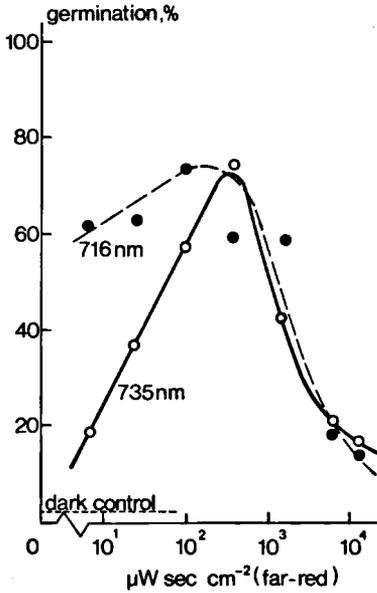
germination,%

Fig. 3. Dose-response curves at two wavelengths.

μW sec cm^{-2}(far-red)

previous paper (BLAAUW-JANSEN & BLAAUW 1975, fig. 1) we demonstrated the same reduction of red sensitivity in 'Noran' seeds if dormancy was induced by imbibition under far-red light during 22 hours at 22°C. Obviously the same effect is obtained by a few seconds of far-red light in 37°C-imbibed seeds as by many hours of far-red at 22°C.
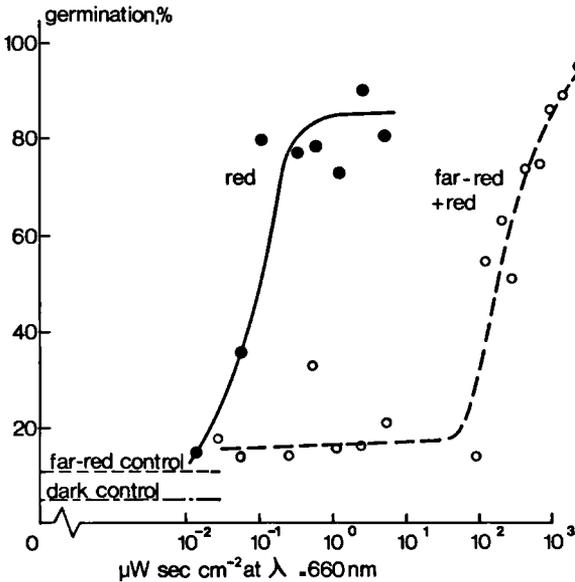
germination,%

μW sec cm^{-2}at λ .660nm

Fig. 4. The shift of the dose-response curve caused by a supraoptimal inhibiting dose of far-red light.

4. DISCUSSION

In an earlier paper the presence in thermo-dormant lettuce seeds of a light-sensitive system with a very low response threshold was demonstrated (BLAAUW-JANSEN & BLAAUW 1975). The action spectrum of this system appeared to be slightly shifted towards longer wavelengths as compared to the normal phytochrome action spectrum (BLAAUW-JANSEN & BLAAUW 1976). This implies an abnormal high inductive effect of far-red light as is confirmed in this investigation.

Now we would like to emphasize the following results from our experiments: the dose-response relation for the effect of far-red light has the shape of an optimum curve (fig. 2A);

by irradiation with supraoptimal inhibiting doses of far-red the highly sensitive system is transformed into a phytochrome system with a response threshold 10^4 times as large as that of the sensitive system (fig. 4) and with the usual action spectrum.

From the above experiments it might be concluded that the light-induced germination of thermo-dormant seeds is mediated by an uncommon form of P_r which is transformed into the usual P_{fr} by small doses of far-red irradiation. Thus the descending part of the optimum curve in our opinion represents the normal reversing effect ($P_{fr} \rightarrow P_r$) of far-red light on the P_{fr} formed from the sensitive system by lower doses of far-red irradiation.

It might be possible that P_{fr} formed from normal P_r differs from P_{fr} formed from abnormal P_r. Evidence for this supposition can be found in the dose-response curve for red-induced germination of the thermo-dormant seeds of *Lactuca sativa* cv May-Queen (BLAAUW-JANSEN & BLAAUW 1975, fig. 2). It was suggested then that the minimum present in the obtained dose-response curve might be due to the interference of two P_{fr} forms at the reaction centres (cf the discussion of that paper).

An optimum curve for the effect of far-red light has also been found by HAUPT & SEITZ (1967) namely for the light-induced movement of *Mougeotia* chloroplasts. HAUPT (1968) worked out this finding by studying the interaction of far-red and red irradiations on the chloroplast movement response. The results of our experiments are in good agreement with his results which can be summarized as follows:

a. the dose-response curve for far-red is an optimum curve (our *fig. 2A*);
b. if red irradiation is preceded by far-red, the far-red effect appears as a shift of the dose-response curve to higher induction energies (our *fig. 4*);
c. a suboptimal induction by red is increased or cancelled by following far-red in the energy range of induction depending upon whether a preirradiation with inhibitory far-red is not given or given (confirmed for the germination of lettuce seeds, to be published).

Haupt considered these effects of far-red light to be caused by the special properties of the *Mougeotia* cell. SEITZ (1973), however, comparing the interaction

of red and far-red light on the germination of lettuce seeds to the results of Haupt, showed a general agreement between the reactions of seeds and *Mougeotia* to far-red irradiation. However, he did not find an effect of far-red by itself on germination and he did not investigate a possible shift of the red dose-response curve by far-red preirradiation. As our experiments clearly show that lettuce seeds and *Mougeotia* chloroplasts respond in the same manner to far-red irradiation, Seitz' opinion that the effects of far-red light are a general characteristic of the phytochrome system, not restricted to the *Mougeotia* cell, is confirmed On this point we would like to recall the shape of the dose-response curves for red light influenced growth of *Avena* mesocotyles and coleoptiles (BLAAUW et al. 1968), for red-light induced anthocyanin synthesis in *Sinapis* seedlings (BLAAUW-JANSEN 1974) and for red-light enhanced geotropic responses of *Avena* coleoptiles (BLAAUW & BLAAUW-JANSEN 1973). These dose-response curves show a stepwise enhancement of the effect of red light with increasing light doses. The first step can be produced by far-red light as well as by red light. The second rise of the dose-response curve occurs at a red dose 10^3 to 10^4 times as large as the dose inducing the first rise. In our opinion the first rise of these dose-response curves is mediated by the uncommon form of P_r present also in thermo-dormant lettuce seeds; the second rise by normal P_r.

The occurrence of the abnormal P_r might also explain the results of RAVEN & SHROPSHIRE (1975). They report on the influence of preirradiation with red or red-far red light on the fluence-response curves for phytochrome control of chlorophyl formation in *Pisum sativum*. In this case too preirradiation caused a shift of the red-dose-response curve with a factor 10^4 and a steepening of the dose-response curve (cf. BLAAUW-JANSEN & BLAAUW 1975, fig. 1).

Moreover, the ratio of the effectiveness of 660 nm to 735 nm for the induction of rapid chlorophyll-a accumulation in non-preirradiated pea appeared to be c. 300 (RAVEN & SPRUIT 1972), the same ratio as was determined for the growth inhibition of *Avena* mesocotyls (BLAAUW et al. 1968) and for induction of germination in thermo-dormant lettuce seeds (this paper). This too suggests mediation by the same pigment.

In our next paper the information contained in the observation of a difference in shape and in steepness of the shifted and the non-shifted dose-response curves will be elaborated.

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