Studies on the physiology of *Funaria hygrometrica* spore germination

Part IV. The effect of blue and red light on the germination of spores

J. KRUPA

Department of Plant Physiology, Higher Pedagogical School Cracow, Poland

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Abstract

The effect of radiation with wave-lengths 654 nm and 425 nm on the germination of *Funaria hygrometrica* spores has been studied. The wave-length bands used for the radiation treatment were employed for various durations and in various combinations. The effects of red and blue light were different in the individual phases of germination when after an inductive radiation treatment light in the 425 nm wave-length was supplied. Differences in the effect of red and blue light are observable in the "post-effects" and in the germination morphology.

INTRODUCTION

The dependence of moss on the wave-length of light needed for germination has been established and described many times. There are two bands of visible wave-lengths that have basically different effects on this process. The action of the red light in the germination process of moss and fern spores was shown to be associated with the phytochrome system (Bauer and Mohr 1959; Mohr and Ohlenroth 1962; Mohr 1963; Mohr, Meyer and Hartman 1964; Steiner 1964; Valanne 1966; Hartman 1967; Krupa 1965 and others). The influence of this system on the various developmental stages during germination varies and it is undoubtedly associated with other photochemical systems as for example with photosynthesis during the II phase of germination (Mohr, Meyer and Hartman 1964; Krupa 1964, 1965, Valanne 1966). None the less there are suggestions, that photoreception of the short wave-lengths needs not to be associated with phytochrome (Hartman 1967; Haupt 1967; Davis 1968).

The influence of light on the germination process depends to a large extent on the duration of spore swelling in darkness. The increase in sensitivity to light caused by the "initial swelling" is not associated with a change in the intensity of metabolic processes (Krupa 1965)

The differential and often antagonistic effects of radiation by short and long wave-length bands has been observed not only on the spore germination process (Klebs 1917; Orth 1937) but also in several other processes as for example during the development of the prothallus in ferns (review in Mohr 1965; Payer and Mohr 1969).

The establishment of differences in the effects of blue and red light on the germination of *Funaria hygrometrica* spores was the subject of the present investigation. The use of light doses that were comparable in energy but used in various radiation programs permits not only to establish the light requirements of the various phases of germination but also to infer indirectly about the nature and photomorphogenetic activity of the reaction systems.

MATERIALS AND METHODS

For the studies spores of *Funaria hygrometrica* were used. The culture conditions, methods of sowing and the apparatus used for the radiation treatments have been described earlier (Krupa 1965; 1967). The spores, after sowing on a Mohr medium solidified with agar have been subjected to monochromatic radiation immediately after sowing or after 24 hours of keeping the material in complete darkness. The radiation needed has been obtained by using 425 nm and 654 nm interference filters ($\frac{1}{2}$ max). Basically 6 radiation variants have been employed. The program of radiation in the individual variants was as follows (see also the results):

1. treatment of the spores immediately after sowing with a radiation in the 425 or 654 nm bands, for various periods of time, after which the percentage of germinated spores was recorded.

2. exposing the spores to 24 hours of radiation after 24 hours of keeping the spores in darkness, followed by recording the germination percentage.

3. after 24 hours of initial spore swelling in darkness they were exposed to radiations for various periods of time and then again the cultures were transferred to darkness for 24 hours, after which the germination percentage was recorded.

4. after 24 hours of keeping the spores in darkness they were exposed to successive periods of blue and red light or vice versa, the doses being equal in duration and energy, after which the germination percentage was recorded.

5. the same radiation treatment as under 4 but followed by transfer for 24 hours to darkness before the germination percentage was recorded.

6. after 24 hours of initial spore swelling in darkness the spores (have been) exposed to blue light at $1200 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ and to red light at $300 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ simultaneously for 24 hours and then the germination percentage was recorded.

For the expositions described in variant 6 an apparatus consisting of two such optical systems as are described above was used. The systems were fixed on to a common plate, at a small distance from each other and at a certain angle. The angle between the optical axes of the system have been so chosen as to allow the light beams to meet in the place where the spores were positioned for exposition. As a result of such an arrangement, in the plane of spore radiation a single light spot was obtained which consisted (after introduction of the appropriate interference filters in the line of the two light beams) of light in the 425 nm and 654 nm wave lengths. The intensity of the 425 nm radiation was $1200 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ and of the 654 nm radiation $300 \text{ ergs cm}^{-2} \text{ sec}^{-1}$. The measurement of the light intensity was made separately for each of the wave lengths and was regulated by changing the voltage of the current supplied to the bulbs.

The percentage of germinated spores has been recorded according to two criteria: criterion I concerning morphological changes described by Mohr (1965) and criterion II, corresponding to the second phase of germination as described earlier (Krupa 1965).

RESULTS

The exposition of *Funaria hygrometrica* spores to radiation of the 425 nm and 654 nm wave lengths has various effects on the process of germination. The results presented in diagram 1 and in graph 2 indicate the percentage of germinated spores scored according to the two criteria. The germination of spores in the 425 nm wave length depends very much on the duration of the exposition (Fig. 2). Exposition for 24 hours immediately after sowing to a blue light at $300 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ induces distinctly the germination according to criterion I but germination according to criterion II has not been observed. Extension of the exposition time to 48 hours causes an increase in the germination percentage according to criterion I to 38.2% and according to criterion II to 10.3%. Spores that have been exposed to 72 hours with a radiation in the 425 nm wave length germinate according to criterion II to a twice as large a percentage as was the case after a 48 hours exposition, while the percentage of spores germinated according to criterion I was 57.3%. When the exposition to 425 nm wave length was preceded by 24 hours of

Fig. 1. Germination of *Funaria hygrometrica* spores in blue and red light used in various programs of expositions: 1 — darkness (24 hrs.); 2 — red light (at 300 or 1200 $\text{ergs cm}^{-2} \text{sec}^{-1}$); 3 — blue light (at 300 or 1200 $\text{ergs cm}^{-2} \text{sec}^{-1}$)

Fig. 2 The percentage of germinated spores according to criteria I and II: A — course of the process using a continuous exposition to light immediately after sowing; B — course of spore germination exposed to light after a 24 hour period of swelling in darkness; red light (654 nm), blue light (425 nm).

keeping the spores in darkness the percentage of germinated spores increases compared to the variant without the pretreatment with darkness. The percentage of germinated spores according to criterion II was twice as large as in the experiments where a 48 hours exposition was applied immediately after sowing. Extension of incubation time for a further 24 hours of darkness after the light treatments does not introduce any basic changes in percentage germination according to criterion II and according to criterion I the percentage increases only slightly (by 7.7%). Reduction to 3 hours of the time of exposition to blue light at $300 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ in the above mentioned variant of the experiment distinctly lowers the percentage germination according to criterion I (by 36.6%) and according to criterion II the germination percentage falls to zero.

As a further modification of the above mentioned variant use was made of blue light of $1200 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ intensity for exposition of 6 hrs. following a 24 hours pretreatment of the swelling spores with darkness. A shortening of the exposition time coupled with an increase in light intensity results in a slight lowering of the percentage of germinated spores according to both criteria, I and II, compared with the results following a 24 hours exposition with a light at $300 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ intensity. On the other hand a shortening of the exposition time to 3 hours results in a considerable decline in the germination percentage, to 28% according to criterion I and to 6% according to criterion II.

Radiation in the 654 nm wave length is much more active in the process of spore germination and 24 hours exposition to this light at 300 ergs $\text{cm}^{-2} \text{sec}^{-1}$ results in 30% germination according to criterion II. However even in this light band at the 300 ergs $\text{cm}^{-2} \text{sec}^{-1}$ intensity it is possible to establish a linear relationship between the percentage of germinated spores according to criterion II and the exposition time (Fig. 2).

Spores exposed for 24 hours to a red light at 300 ergs $\text{cm}^{-2} \text{sec}^{-1}$ intensity following a 24 hour pretreatment of initial swelling under darkness germinate to a high percentage (72.7% according to criterion II) and the extension of the incubation time by another 24 hours in darkness causes an increase in the percentage of germinated spores to 91% according to criterion II.

Reduction of exposition time to 6 hours coupled with an increase in the light intensity to 1200 ergs $\text{cm}^{-2} \text{sec}^{-1}$ has a considerable negative effect on germination since it becomes reduced to only 18.5% according to criterion II. This percentage is comparable to that observed following the analogous treatment with blue light. On the other hand the percentage of germinated spores according to criterion I is 30% higher in the experiments where exposition to red light was employed. Reduction of exposition time to 3 hours of 654 nm wave length radiation does not cause any basic changes in the percentage of germinated spores compared with the 6 hour exposition with a 4 times higher light intensity. The data presented in the diagram indicate that the percentage of germinated spores, simultaneously exposed for 24 hours to light of both the wave lengths, is the same as was obtained following exposition for the same duration of time to red light only. In spite of using a 4 times more intense blue light it was not possible to establish any inhibitive effect of this radiation on the germination process. There exist however certain differences in the percentage of germinated spores when the 24 hour long periods of exposition to blue and red light are given consecutively. In these experiments the percentage of germinated spores according to criterion II was higher (by 13.3%) in the experiments where the last exposition was with light at the 654 nm wave length. The inhibitive effect of blue light used after an inductive exposition to light at 654 nm wave length is best seen when the expositions are reduced to 3 hours. The difference after using a light intensity of 1200 ergs $\text{cm}^{-2} \text{sec}^{-1}$ was 16.3% of the germinated spores according to criterion II.

Differences in the methods of action of the two studied wave lengths are observable also in the germination morphology. No anomalies were to be found when the spores were allowed to germinate under light in the 425 nm wave length or under both the 425 and 654 nm wave lengths given simultaneously. However when only the red light was given numerous anomalous forms were observed (Krupa 1967). In the experi-

ments where radiation with 425 and 654 nm wave lengths was supplied consecutively the percentage of anomalies is smaller and their morphology indicates that they occurred during the treatment with red light.

DISCUSSION

Light is a factor that controls a number of developmental processes, among these also the germination of spores in mosses. The action of light in the germination process takes place through a complex system of reactions. For many plants several reaction systems associated with morphogenesis have been described: the photomorphogenetic system associated with photosynthesis, the phytochrome system and the system or reactions associated with the visible light radiation at wave lengths below 500 nm (Mohr, Meyer and Hartman 1964; Mohr 1965; Valanne 1966; Miller and Miller 1967; Jacques 1967; Payer, Striffer and Mohr 1969; Steiner 1970 and others). The participation of the above mentioned reaction systems in the germination process of moss spores has been established several times (Bauer and Mohr 1959; Mohr, Meyer and Hartman 1964; Valanne 1966; Krupa 1967; Payer and Mohr 1969).

The problem is to determine their mutual relationships and their synergistic or antagonistic influences on the germination processes.

The use in the present studies of radiations of the 425 nm and 654 nm wave lengths for the treatment of spores has induced different responses of the germination process.

Analogous effects following the use of blue and red light were observable in the experiments during which the expositions were continuous from the moment the spores were sown. The percentage of germinated spores in these conditions, particularly in the initial stages, depends on the time of exposition. This relation is very distant when radiation of the 425 nm wave length was used, both for criteria I and II in estimating the germination. On the other hand the time effect on the germination of spores exposed to light in the 654 nm wave length is observable only when the germination is evaluated according to criterion II. The differences in response to treatment by these two wave lengths concern the direct effect of these wave lengths on the initiation of germination.

The percentage of germinated spores, which have been subjected to radiation by light in the 654 nm wave length is higher than following the use of blue light at the same intensity. This indicates that there is a relation between radiations of 654 nm and the action of the low-energy phytochrome system. However both in experiments with red light and in those in which blue light was used it was possible to observe a certain inhibition effect on germination following continuous exposition immedia-

tely after sowing, compared with experiments in which a 24 hour period of darkness was supplied preceding the exposition to light of the same type and duration. This can indicate on the one hand that the phytochrome system participates in the effects of both the wave lengths on the germination process (Hartmann 1967; Steiner 1967 and 1969; Jacques 1967 and others) and on the other it shows that the role of the initial swelling of spores in darkness is still not clear (Krupa 1964; Mohr, Meyer and Hartman 1964). The process of initial swelling of spores in the darkness is not associated with changes in the intensity of gaseous exchange however it may be related to activation of some processes that intensify the sensitivity of the moss spores to light (Krupa 1964; Valanne 1966).

Further differences in the reaction of *F. hygrometrica* spores to radiations used in the study concern the post-effects, which are observable only following expositions to 654 nm light. Spores which have first been exposed to blue light and then were transferred for 24 hours to darkness do not germinate to any higher percentage than those which have been counted immediately after the exposition. On the other hand this post-effect is very distinct following expositions to red light. This can only mean, that red light initiates processes that can continue to operate in the darkness. On the other hand in order to obtain positive effects with blue light continuous exposition is required. A similar phenomenon can be deduced from the results of experiments in which the exposition times of spores to red light have been reduced to 6 or 3 hours following an initial period of swelling in darkness. This may confirm the necessity of forming a specified balance in the level of P_{Fr}/P_R necessary for the initiation of morphogenetic reactions (Hartman 1967; Jacques 1967; Steiner 1970). On the other hand as has been shown earlier (Krupa 1967) the germination of spores exposed to radiation in the short wave lengths of the visible spectrum is associated with a high energy reaction. The active photoreceptor in this range is the phytochrome system (Miller and Miller 1967; Hartman 1967; Steiner 1970), though one cannot definitively exclude the possibility that other reaction operates or that there are interactions between the effects of several systems (Haupt 1967; Davis 1968). One of them could be the photosynthetic system. However intensity of photosynthesis in the first stages of germination (determined by comparison with the productivity in white light of similar intensity of radiation, see Krupa 1965) is low and the same at both the wave lengths used in this study. This confirms the data of Payer and Mohr (1969) that the amount of photosynthesis in prothallus of the fern *Dryopteris filix-mas* is the same under red and blue light.

On the other hand of importance is the observation that the amount of protein synthesis is greater under blue light as well as the synthesis

of several other metabolically active substances (Ohlenroth and Mohr 1964; Drum and Mohr 1967; Payer and Mohr 1969; Burns and Ingle 1970 and others).

The diversity of systems associated with the processes of germination as well as their unequal participation in the various phases of germination is indicated by the experiments in which the spores were exposed to 425 nm and 654 nm radiation simultaneously or consecutively during a time of less than 24 hours. The effect of the use of various programs of short expositions does not show up in the second phase of germination after the spores are transferred for 24 hours into darkness. Lack of a definite effect could have been caused by the exposition time being too short to be able to induce changes during the subsequent darkness. However in the experiments of other authors a shorter exposition time with the active radiation resulted in a much higher percentage germination (Bauer and Mohr 1959; Mohr, Mayer and Hartman 1964) which could have been caused by different conditions during exposition or else by different criteria for recording germination.

The differences observed in the morphology of spore germination following exposition to blue and red light used in various radiation programs confirm the observations of many authors concerning the „normal“ development of protonema in blue light. The role of blue light in this process can be demonstrated by using consecutive or simultaneous expositions to light at the two wave lengths employed. Thus it can be assumed on the basis of the results obtained that the range of short and long wave lengths within the visible spectrum act through different physiological mechanisms and the fact that the results are not clear cut could be caused by the different effects becoming superimposed on each other.

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