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Studies on the physiology of germination of spores of *Funaria hygrometrica*

III. The influence of monochromatic light on the germination of the spores

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INTRODUCTION

In the previous studies (Krupa 1964, 1965) the influence of white light on the germination of *Funaria hygrometrica* spores, as well as on some physiological processes taking place during germination (such as water balance, respiration and photosynthesis) was investigated. It is known, however, that the different spectral ranges influence the germination process of spores in different ways (Klebs 1917; Listowski 1927; Stephan 1928; Orth 1937).

Recent investigations by Bauer and Mohr (1959) have shown that the germination of *Funaria* spores is associated with the action of the phytochrome system with its characteristic reversible effect when exposed to near and far infra-red radiation. This system controls the germination of the spores in a great number of mosses and ferns; there are, however, some exceptions in which the influence of light on germination is far more complicated. The influence of monochromatic light on the germination of *Funaria* spores has so far been examined only for wavelengths with a maximum induction activity and for the reaction reversion controlled by the phytochrome, making use only of short impulses of weak radiation (Bauer and Mohr 1959). The widening of the scope of the above mentioned investigations seems indicated for two reasons:

a. As the experiments with white light have shown (Krupa 1965), very low light intensities only induce the content of the spore to turn green and the exosporium to burst, whereas light of higher intensity is required for the further stages of germination. On the germination criterion used by Bauer and Mohr (1959) it is possible to state that the first germination stage depends on the phytochrome system.

How the further stages (e.g. the formation of chloronema) are dependent on the light intensity and wavelength is, however, an open question. This problem is perhaps connected with the high energy reaction of photomorphogenesis (Mohr, Wagner Hartmann 1965).

b. In the reactions controlled by the phytochrome system, different effects of short wavelength radiation (stimulation or retardation) were estabilished. Thus, it

seems desirable to study the influence of the whole spectrum range on the germination process of *Funaria* spores.

Data so far obtained concerning the localization of the pigments active in the morphogenetic systems indicate that the latter are localized within the cytoplasm. The cell-wall of the spore of a brown-yellow color acts as a kind of light filter modifying the energy and spectral composition of the light reaching the active pigments. In the last part of the present work, the optical properties of the cell-wall have been measured to ascertain which wavelengths are absorbed in the cell-wall of the spore and to what extent.

MATERIAL AND METHOD

Spores of *Funaria hygrometrica* were used for the investigation. The material was obtained and seeded according to the previously described method (Krupa 1965). The spores sown in Petri dishes on Mohr's medium solidified with 1% agar were irradiated for 24 hours after seeding with monochromatic light. 110 V-

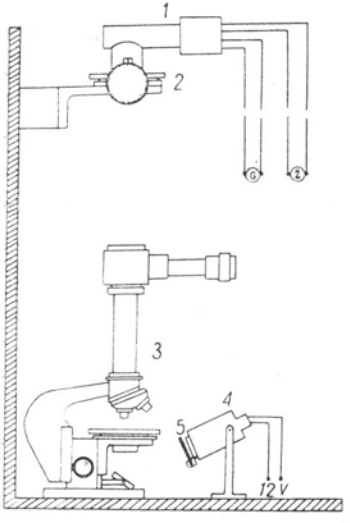

Fig. 1. Scheme of apparatus for measurement of the light absorption through the cell-wall of spores

1 — photomultiplier, feeder and galvanometer; *2* — microscopic stage; *3* — microscope Ng; *4* — microscopic lamp; *5* — interference filter

750 W bulbes were used as sources of light. Monochromatic light was obtained by means of interference filters with transmission nm wavelengths of: 362, 382, 400, 429, 480, 497, 529, 544, 574, 594, 630, 654, 680, 703, 732, 769, 823. Monochromatic light of an intensity of 500 erg/cm^2/sec, 5000 erg/cm^2/sec, 15000 erg/cm^2/sec was used. The regulation of light intensity was controlled by changing the voltage supply of the lamps. The influence of light of 362 nm wavelength and 15000 erg/cm^2/sec intensity was not determined because the impossibility of obtaining such a high intensity at an appropriate time used.

The spores were irradiated by means of the previously described apparatus (Krupa 1965) with an interference filter used instead of glass Schot filter.

Light intensity was measured by means of a photo-cell with a neutral reticular filter

and a diaphragm of 10 mm diameter connected to a Zeiss scale galvanometer. The photo-cell was calibrated in erg/cm²/sec separately for every filter by comparing its readings with those of a termopile (Kipp and Zonnen) of known absolute sensitivity. The calibration of the photo-cell was repeated every few days. Light intensity was measured twice: before and after exposure in view of posible changes in emission of the lamps. In the case of a high emission (over 10%), the measurment was eliminated from further calculations. The spores were observed directly on the Petri dishes and discarded after their germination had been calculated.

The absorbtion of light by the cell-wall of the spores was measured with an apparatus present on fig. 1., located in dark room. The apparatus is composed of a Zeiss Ng microscope with an apochromatic optical system, a microphotographic attachment located over the microscope on a stable stand, a microscopic stage with a black screen (aperture 8 mm dia), a photomultiplier with feeder, a scale galvanometer, and a microscope lamp with a holder for the interference filter. The magnified image of the object was obtained on the photo-cell plane of the photo-multiplier with the aid of the optical system (microscope, microphotographic objective). The photomultiplier was fixed to the microscopic stage so as to allow its shifting in to two different positions. In one of these the photo-cell was illuminated by the light which has passed through the spore walls, in the other only the intensity of the light passing through the glasses and a thin layer of water was measured. The aperture in the black screen supporting the photo-multiplier made it possible to limit the area of the microscopic image sector, from which light was directed to the photo-multiplier. The horizontal plane of the stage was perpendicular to the optical axis of the microscope. The stage had a shifting hand-wheel of 20 cm dia. with an accurate scale, permitting a return to the same spot after a change of position. The cell-walls used for the transmission measurments were taken from spores kept for 16 hours in water and under light. After this time the spores were placed on a slide and crushed by strong pressure on the cover slide. The exuded contents of the spores was removed by repeated rinsing with water under the slide. The obtained preparation was sealed with vaseline along the edge of glass cover slide to prevent dessication. After placing the preparation on the microscope stage readings were made of the intensity of monochromatic light passing through 2 walls of the spore, as well as of light passing through the base and cover slide and the layer of water after the photomultiplier had bee shifted to another possition. These measurments enabled the calculation of the percentage of light of known wavelength absorbed by the 2 layers of the spore exosporium. Absorption was computed for one wall according to the formula $T_x = /T_1/^d$, where T_x — transmittance of a double exosporium layer, T_1 — transmittance of single layer, $d = 2$.

RESULTS

The influence of monochromatic light on the germination of spores was investigated in the range 362—823 nm. The results presented in fig. 2, show the percentages of germinated spores after a 24 hrs irradiation with monochromatic light

of the intensities: 500, 5000, 15000 erg/cm²/sec. Light in the range from 580 to 700 nm was found the most active radiation for the germination process, and the maximum activity is to be found at about 680 nm of the light wavelength. The percentage of germinated spores falls sharply in the range of 700—823 nm, and for an intensity of 500 erg/cm²/sec and a light wavelength of 823 nm amounts to 0.4%, while for a light intensity of 15000 erg/cm²/sec — it is 16%.

The germination of spores in the short-wave spectrum range (362—500 nm) shows a marked dependence on the light intensity. Light intensity of 500 erg/cm²/sec

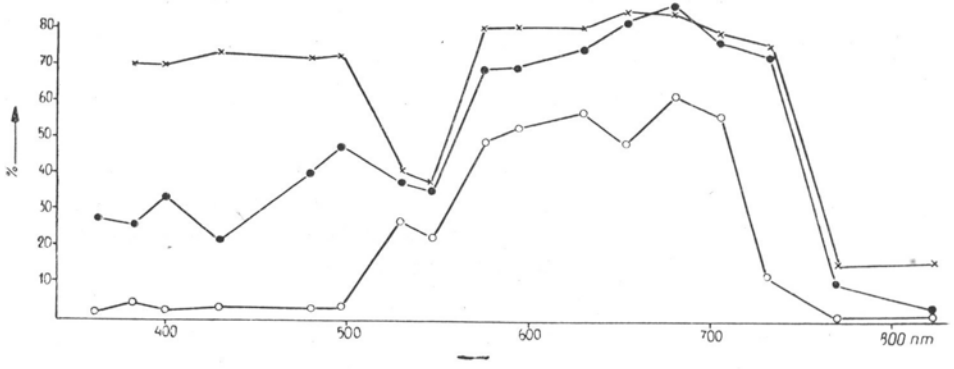

Fig. 2. Germination of spores after a 24 hrs irradiation with monochromatic light: —o—500 erg/cm²/sec, —●— 5000 erg/cm²/sec, —x— 15000 erg/cm²/sec.

brings about the germination of 0 to 0.38 % of spores, while on the average 30% germinate under an intensity of 5000 erg/cm²/sec 70% of spores irradiated for 24 hrs with light of an intensity of 15000 erg/cm²/sec germinated. As shown by the form of curves on fig. 2, the percentage of germinated spores decreases in the radiation range between 500 and 544 nm wavelength. This decrease in the percentage of germination is particularly clear at the intensities of 5000 and 15000 erg/cm²/sec. The percentage of germinated spores under a light intensity of 15000 erg/cm²/sec is lower by about 31% in comparison with the germination percentage in the previous radiation wavelengths. It has been presumed that the decrease of germination percentages in this range, as well as the lack of full germination at high radiation intensities, my be caused by the inhibiting action of this light colour on germination. To check this supposition the spores were irradiated for 18 hrs with red light (range 680 nm) of an intensity of 15000 erg/cm²/sec and then kept in darknes for 24 hrs after which the computed percentage of germinated spores amounted to 59,6%. In a second experimental series, the spores were irradiated (after an 18 hrs dose of 680 nm light) with light of 529 nm wavelength and an intensity of 15000 erg/cm²/sec for 24 hrs. 89.6% of spores treated in this way germinated. In a similar experiment when light with a wavelength of 654 nm was used for primary induction, the percentage of germinated spores amounted to 50, after 18 hrs of irradiation and 24 hrs in darkness. However, when the spores instead of being

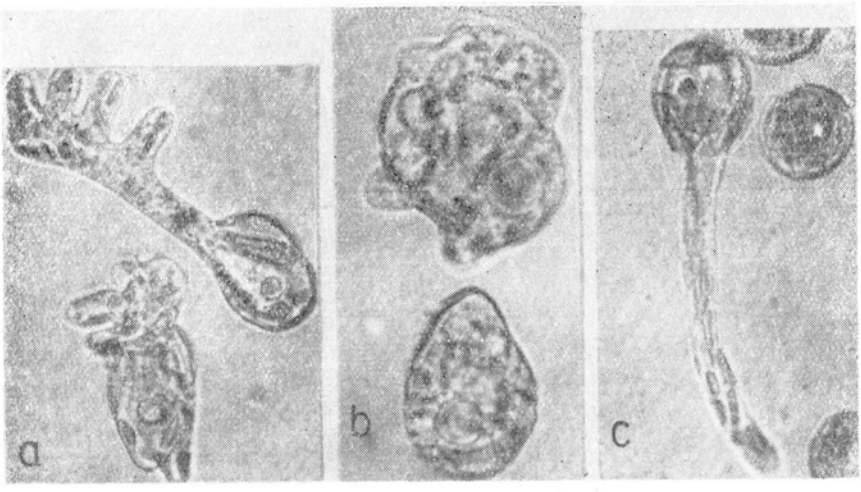

Fig. 3. Spores germinated: *a* and *b* — in monochromatic light of the spectral range from 600—823 nm, *c* — in short-wave range of the spectrum (362—500 nm).

kept in darkness for 24 hrs were submitted for the same time to irradiation by light in the range of 544 nm and an intensity of 15000 erg/cm²/sec, the percentage of germinated increased to 88.5%. Therefore radiation in the critical wavelength range does not inhibit the process, and the low percentage of spores germi-

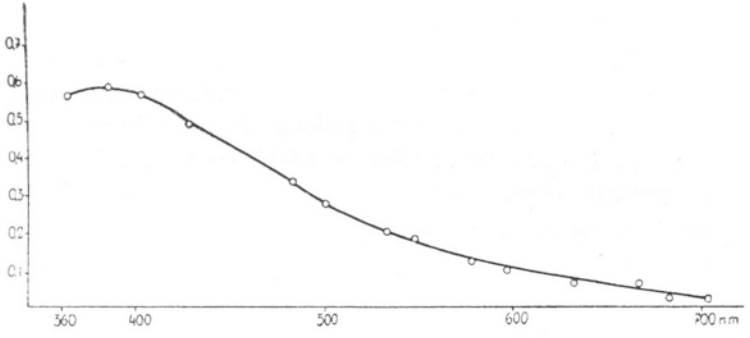

Fig. 4. Absorption of monochromatic light through the cell-wall of the spore. *x*-axis — wavelength in nm, *y*-axis — relative absorption.

nation cannot be attributed to the antagonistic action of this wavelength range in relation to the germination stimulating effect.

Differences between the influence of the short-wave (362—500 nm) and the long-wave radiation range, are to be seen not only in the number of germinated

spores, but also in the germination morphology (fig. 3). As microscopic observations showed, the chloronema produced during irradiation with short-wave light is 2—3 times shorter than the chloronema grawn in the long-wave range, and its form is thready and unbranched. On the contrary, spores germinate abnormally in the long-wave range of the visible spectrum the chloronema often producing branches or thickenings. In many cases the growth of the chloronema length appears to be inhibited, and the chloronema threads develop into shapeless forms. No anomalies in the development of the rhizoid were observed. *Funaria hygrometrica* spores have quite thick coloured exosporium. The brown pigment present in the exosporium acts as a light filter, restricting the penetration of respective spectrum ranges to the protoplast. To determine what proportion of the light penetrates to the intensior of the spore, the cell-wall absorption was measured in the respective light wavelengths. The results presented in fig. 4., show that short-wave light in the range 362—400 nm is absorbed most, maximum absorption occurring at about 380 nm. Absorption decreases together with the increase of the wavelength, and reaches values close to zero at 700 nm.

DISCUSSION

In sufficient attention has so far been paid to the influence of light absorption in certain pigments in the cell-wall of spores which modifies the kind of light penetrating to the active pigments. As results from data obtained in the present work (cf. fig. 4.) short-wave light is attenuated 2—3 times after passing through a single cell-wall layer, yet long-wave radiation is hardly absorbed at all. Therefore it may be assumed that the activity of short-wave radiation is in reality 2—3 times greater than results from experiments in which the same illumination energy is applied in the short and long-wave range. This should be taken into account in estimating the results of previous investigations an the influence of continuous light on germination (Klebs 1917; Listowski 1927; Orth 1937) as well as the results of the present work.

The germination curves as a function of the light wavelength (fig. 2.) show a different course from those which were determined for low-energy reactions controlled by the phytochrome system (Mohr 1956). It is true that in both cases red light of 600—700 nm brings about the highest germination stimulating activity, nevertheless no inhibiting action either of the short wave, or of the far red range was observed. In particular, high illumination intensities induce high germination percentages both at 732 nm, and in the range of 362—500 nm. If the reduction of the short-wave light intensity due to absorption in the exosporium is taken into consideration, then the activity of this range at high light intensities is comparable with the activity of long-wave radiation, Mohr, Meyer and Hartmann (1964) noticed a similar phenomenon in the work of *Osmunda* on the process of rhizoid generation hence inferring the importance of photosynthesis in this process. Attention

has already been paid to the presumable influence of photosynthesis on the germination of *Funaria* spores, in experiments with white light (Krupa 1965).

However, even assuming that photosynthesis is the cause of the germination of *Funaria* spores it is hard to explain the high germination percentage under light of 732 nm wavelength because photosynthesis does not take in this range. On the other hand morphotic reactions controlled by the high-energy photosystem (HER) are known and are characterized by a linear dependence on the intensity up to high energy light and active spectrum with a maximum effect between 440 and 470 nm, as well as 640 and 730 nm (Hendricks and Borthwick 1965).

It is difficult to explain the considerable decrease of germination percentage in the range of 529 and 544 nm, as well as the phenomenon of light saturation of the germination process at a level of barely 50% in comparison with the remaining ranges. Assuming that photosynthesis is responsible for germination, it may be presumed that chlorophyll synthesis as well as light absorption in photosynthesis are in considerable in the above mentioned spectrum range, and that the low germination percentage is the result of the cumulative effect of both processes. Such a conception may be confirmed by the fact, that a temporary dose of 529 or 544 nm radiation applied after an inductive dose of red light increases the germination percentage by approximately the amount that would be caused by continuous illumination in the range mentioned above (abt. 30%). It may also be supposed however, that in the above mentioned range, absorption by one of the germination controlling pigment comes to its end, while absorption by the second system begins. Low absorption in both systems may be the cause of a considerable decrease in the germination percentage.

On the basis of the results obtained, it is difficult at present to decide which one of the two processes, photosynthesis or HER is responsible for the germination of *Funaria* spores in the later stages. The two different photomorphotic systems present in the cell, may supplement each other mutually in natural conditions in the morphogenetic reaction to light. Whereas long-wave radiation absorbed by one of the systems brings about an increase in the cell growth and may lead to anomalities in development, short-wave radiation on the other hand, absorbed by the second system acts as an inhibitor on these processes. Anomalous morphological changes in germination observed in long-wave radiation, indicate the existence of two absorption systems, and perhaps the different action of short- and long-wave light. Their appearance cannot be attributed to the influence of light intensity because they are to be observed in all three cases. The obtained anomalites are similar to those described by Wettstein (1953) under the influence of vitamin B_1, IAA and colchicin. On the other hand no anomalities in germination morphology were observed when short-wave (362—500 nm) radiation was used, in any of the applied light intensities. The growth of protonema is much weaker in blue light then under long-wave radiation. Mohr and Ohlenroth (1962) reported a similar effect for the gametophytes of *Dryopteris felix-mas*, fern whose „normal" development was caused by blue light, while red radiation brought insignificant elongation of thready cells and a slowing down the tridimensional gametophtes growth.

SUMMARY

1. The influence of the monochromatic light of different wavelength on the germination of the Funaria hygrometrica spores, was determined.

2. Two ranges influencing germination in different ways were distinguished. The short-wave (362—500 nm) light range induces marked germination only after applying higher intensities (above 500 erg/cm^2/sec), while long-wave radiation (580—700 nm) brings about distinctly visible germination at considerably lower intensities.

3. Long-wave radiation, moreover, causes the appearance of anomalities in the morphology of the chloronema growing under this light.

4. The absorption of monochromatic light by the cell-wall of the spore was investigated, thus permitting the determination of the amount of light penetring to the protoplasm.

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Badania nad fizjologią kiełkowania zarodników Funaria hygrometrica

Cz. III. Wpływ światła monochromatycznego na kiełkowanie zarodników

Streszczenie

1. Określono wpływ poszczególnych zakresów światła monochromatycznego na kiełkowanie zarodników *Funaria hygromatrica*.

2. Wyodrębniono dwa zakresy wpływające w różny sposób na kiełkowanie. Zakres światła krótkofalowego (362—500 nm) wywołuje wyraźne kiełkowanie dopiero po zastosowaniu większych natężeń (powyżej 500 erg/cm^2/sek), zaś promieniowanie długofalowe (580—700 nm) wywołuje wyraźne kiełkowanie przy użyciu znacznie niższych intensywności.

3. Promieniowanie długofalowe wywołuje ponadto powstanie anormalności w morfologii chloronemy wyrastającej w tym świetle.

4. Zbadano absorpcję światła monochromatycznego przez błonę zarodnika, która pozwoliła na ustalenie ilości światła docierającego do protoplazmy.