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Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*

II. Studies with polarized light

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Polarized light was applied succesfully in the study of orientation of photoreceptors in germination of spores and zygotes (Jaffe 1956, 1958; Jaffe and Etzold 1962, 1965; Mayer zu Bentrup 1963, 1964) and in growing of fern prothalia and fungi hyphae (Bünning and Etzold 1958; Etzold 1961, 1965, Shopshire 1959). Haupt and his collaborators were able to detect the orientation of phytochrome molecules in *Mougeotia* and *Mesotenium* by studies in polarized light (Haupt 1960, 1962; Haupt and Thiele 1961; Haupt and Bock 1962; Schönbohm 1965). Recently Mayer found that the characteristic orientation of chloroplasts in *Selaginella* can be induced by illumination with polarized light.

The aim of the present study was to draw some conclusions about the orientation of photoreceptor molecules in the cell of *Funaria hygrometrica* on the base of the behaviour of chloroplasts under illumination polarized with radiation.

METHOD

Leaves of *Funaria hygrometrica* placed on the slide in water medium were illuminated under microscope. In the first part of the study blue, not monochromatical, light was used. This light was obtained by application of filters GG 18/2mm+ +BG 1/2 mm+$CuSO_4$ solution 40 g/l/5 cm. Relative transmission of the filter set is illustrated in fig. 1. Regulation of light intensity was performed by use of a neutral filter (Schott — NG). Measurement of light intensity and registration of chloroplasts arrangement had been previously described (Zurzycki 1961, 1962).

For the study in monochromatical radiation an interference filters or UV set described in the first part of this study were used (Zurzycki 1967). The polarizing element was introduced in the light path before the microscopic condensor.

Three kinds of polarizers were used: 1. polaroid filter „Bernotar" (Zeiss) — for illumination with not monochromatical blue light, 2. Glan-Thomson prism — for irradiation with monochromatical light in the visible and near UV range and 3. Nikol prism sealed with glycerine for UV equipment. The spectral transmission of the polarizers is presented in fig. 1 The transmission of the linear polarized radiation by the polarizers oriented in a „crossed" position was for 1 and 2 below

1% in the whole range between 600 nm and the short wave lenght limit of transmission, for 3 in the far UV (at 266 nm) only, about 20% of the radiation transmitted in a „parallel" position. (Fig. 1).

Circular polarized light was obtained by use of 1/4 and 3/4 λ mica plates put on the Glan Thomson prims at the 45° in respect to the plane of polarization.

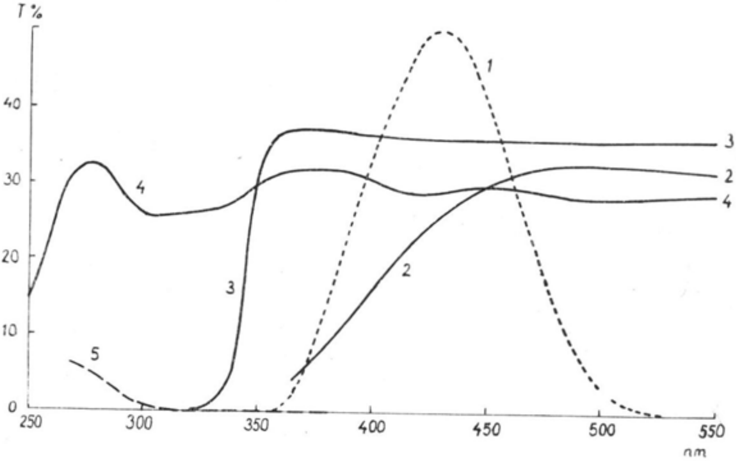


Fig. 1. Relative transmission of the filter set used for illumination with nonmonochromatic blue light (*1*) and the transmission of polarizers: polaroid filter (*2*), Glan-Thomson prism (*3*) and Nikol prism (*4*). Transmission of the Nikol prism in a cross position to the plane of polarization (*5*).

Cinematographic pictures were taken on a 16 mm film with the time laps method. Subsequent frames were exposed every 12 sec. which gave the acceleration of movements of chloroplasts 300 x.

RESULTS

When a leaf of *Funaria* with chloroplasts in the low light arrangement is illuminated with high intensity of polarized light the displacement of chloroplasts which takes place depends on the direction of polarization. If light is vibrating perpendicular to the leaf axis (i.e. parallel to the shorter walls of the cell) the chloroplasts displacing to the side walls avoid the walls parallel to the vibrating plane. They situate themselves on the longer side walls leaving the shorter ones quite free (fig 8*a* — plate I). In the enough high light intensity all chloroplasts take a profile position. By illumination with light vibrating parallel to the longer axis of the cell most chloroplasts displace themselves to the shorter side walls and gather there forming a dense layer but a part of them usually remain near the center of the cell in a flat position. Even in high light intensity, which causes full displacement in the former case no complete high light arrangement was obtained (fig. 8b, 9 — plate I). The percent of chloroplasts remaining in the flat position depends on the geometrical shape of the cell. In the average it is about 40%. The longer and narrower the cell, the greater is this value. For extremely long and narrow cells' the percent of remaining chloroplasts may reach 70, for the cells of a square like shape it may fall to 0. In lower light intensities only partial high light intensity arrangement is reached even when the vibrating plane of light is perpendicular to the longer cell axis, but the general pattern of arrangement is the same i.e. gathering of chloroplasts on these cell walls which are perpendicular to the vibration plane (fig 10 — plate II).

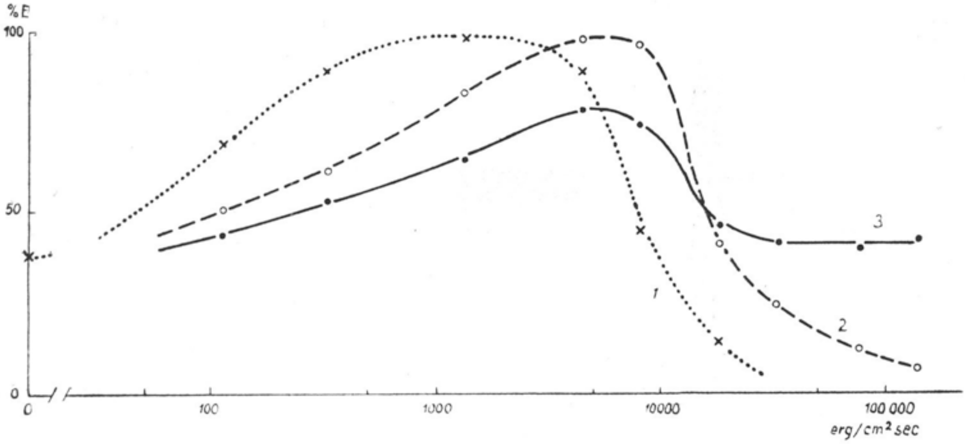


Fig. 2. Dependence of chloroplasts arrangement (Y axis — %E) on the light intensity. Starting position — low light arrangement, time of irradiation 90 min. Curve *1* for unpolarized light, *2* and *3* for polarized light vibrating parallel (*2*) or perpendicular (*3*) to the longer cell axis.

By illumination with a not polarized light full low light position of chloroplasts can be obtained or maintained in a broad range of radiation intensities. On the contrary, by illumination with polarized light the low light arrangement can be obtained in a very narrow range of light intensity and even then some percent of chloroplasts remain on the side walls. This is especially the case when light is vibrating perpendicularly to the longer axis of the cell.

If light intensity is further lowered the pattern of arrangement taken by chloroplasts changes reversely to the former one. Some chloroplasts remain on the upper and lower cell walls and the others gather on these side walls which are now parallel to the vibrating plane (fig 11 — plate II).

In fig 2 the dependence of the final arrangement of chloroplasts on the light intensity is presented for both directions of polarization. On the same graph a similar dependence of chloroplasts arrangement on the intensity of non polarized light of the same spectral quality is shown. By comparison of these curves it is clearly to be seen that the intensities of polarized light which bring highest low light arrangement cause the partially high light displacement when applied in a non polarized form. The threshold of the reaction is shifted to the higher intensities. It other words, in the high intensity region of light the chloroplasts are 1,5—2 times more sensitive to non polarized than to polarized light of the same spectral quality.

It should be noticed that the geometrical details of the cell walls have a significant influence on the arrangement taken by chloroplasts in polarized light. In the case of a vibrating vector perpendicularly to the longer axis of the cell, the place suitable for chloroplasts on the shorter side walls is rather limited in comparison to the amount of chloroplasts in the cell. They occupy these walls in a dense layer, but if any of them remain on the longer side walls so always in the neighbourhood of the shorter wall of another cell. (Fig. 3). On the contrary by placing themselves on the longer side walls which offer much more place — the chloroplasts avoid the curvatures of the side walls near the shorter walls. The place close to side wall of the neighbouring cell is also very often free (Fig. 3).

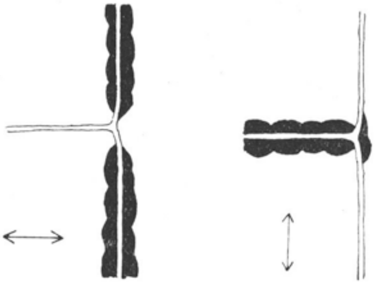


Fig. 3. Scheme of chloroplasts arrangement by the side walls. Explanation in text.

Differences which appear in the absorption in the photoreceptor after applying differently directed polarized light may also exert an influence on the kinetic of the chloroplasts movements. This problem was studied by two methods: determining of the mean percent of chloroplasts beeing in flat position during the displacements and by cinematography. The first method showed that there are no significant di-

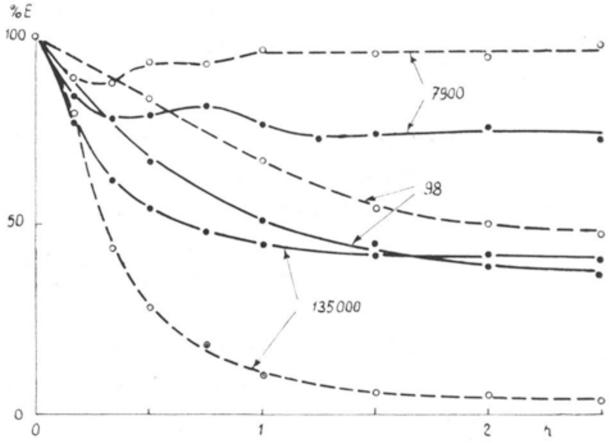


Fig. 4. Time course of the dispalcements. Starting position — low light arrangement. Numbers denote intensity of blue light in erg/cm² sec. Light vibrating parallel —●—●— or perpendicular - - ○ - - to the longer cell axis.

fferences in speed of changing the %E in time between displacements caused by both directions of polarized light especially in the first phase of displacements. In the later period differences arise and are connected with the impossibility of reaching full high light arrangement in one case (Fig. 4).

The result of film analysis of the movements of chloroplasts in one cell is presented in fig 5. In the same cell the low light → high light rearrangement was studied.

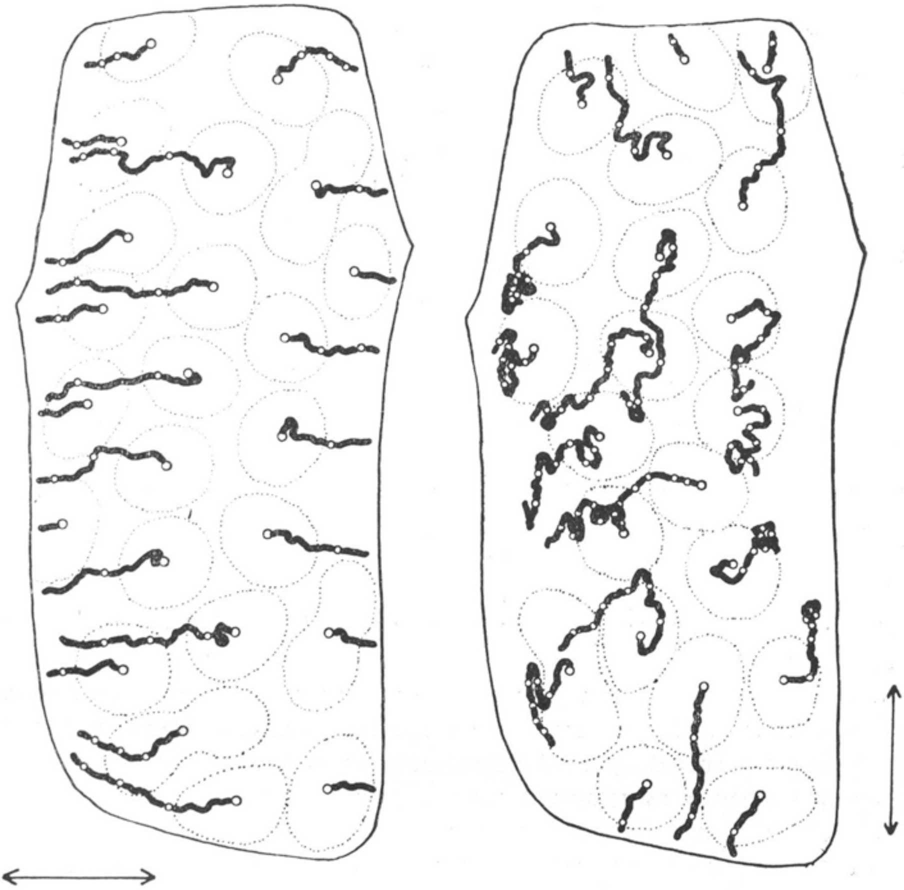


Fig. 5. Movements of chloroplasts in the same cell under influence of polarized blue light of the intensity 96000 erg/cm^2 sec. Curves show the paths of the central points of chloroplasts; white circles — position of the center every 10 min.

The low light position was caused by not polarized light of 1200 ergs/cm^{2}sec. intensity. Strong light has the intensity 96 000 ergs/cm^{2}sec. and was polarized either perpendicularly (in case *a*) or parallel (as in case *b*) to the longer cell axis. As it can be seen there is a predominance of the direction of chloroplasts movements in the vibration plane of light. This predominance however is clearly pronounced in the neighbourhood of side walls which in the given case preferred for the chloroplasts.

In the first experiment (*a*) all chloroplasts move in this direction, in the other (*b*) only chloroplasts situated near the shorter side walls. These occupying the central part of the cell show irregular movements with frequent changes in direction and most of them do not leave the upper cell wall. In both cases even when the chloroplasts come to the „forbidden" side wall they do not creep on it but move along the edge of the cell till they reach the proper place (*a*) or if this is impossible show erratic movements near this edge for a longer time. No distinct differences in the mean velocity of chloroplasts can be noticed between case *a* and *b*.

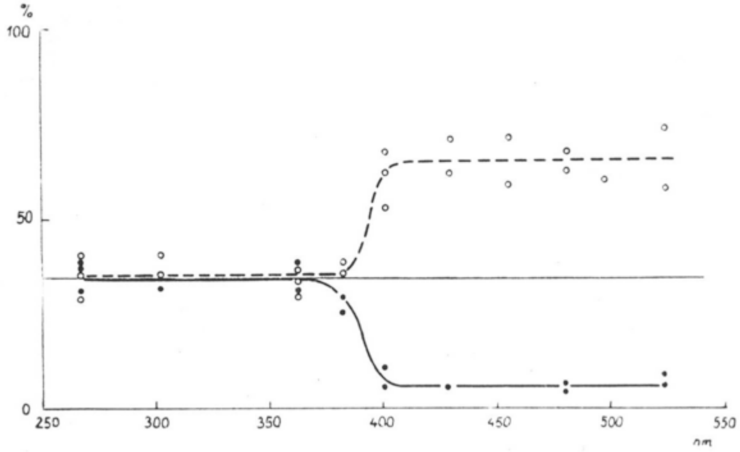


Fig. 6. Dependence of the percent of chloroplasts remaining in profile position on the shorter cell walls on the wave length of radiation after 90 min irradiation with polarized radiation. Explanation in text.

Illumination with left or right rotating circularly polarized light gave no characteristic features. The final arrangement and the speed of displacement were the same as in not polarized light. No differences either in the sensitivity of chloroplasts to the light intensity as compared to not polarized light could be noticed.

The above described experiments were made with not monochromatical blue light. In the last series of experiments polarized light obtained by the use of interference filters or monochromator was used. The starting position was a dark arrangement, because the intensities of radiation obtainable in UV were too low to induce low light → strong light displacements. It was stated that the arrangement typically depending on the direction of polarization may be obtained only in the visible range of radiation. Polarized near and far UV induce only displacements typical of low light intensity but accumulation is not privileged on either of the side walls. The results of irradiations are illustrated in plate III.

A following method was applied for a quantitative determining of directionary influence of polarized radiation: Cells with a dark arrangement of chloroplasts were irradiated with an intensity causing about 50% E (40—60%) by 1,5 hour. The intensity varies distinctly according to the wave length (Zurzycki 1967). After irradiation was finished the number of chloroplasts remaining on the side walls was counted separately for the longer and shorter side walls. It was found that determining of this number is possible with no great error by illuminating the microscope with light 480 nm, which is well enough absorbed by chloroplasts to make them clearly visible but not so high as to allow the distinction of two chloroplasts lying one over the other. Assuming all chloroplasts remaining on the side walls as 100%, the percent of those laying on the shorter cell walls was calculated. The mean ratio of the length: breadth of *Funaria* cell as measured for 100 cells is 1 : 0.525. That means that when there is no priviledge of any side cell wall and the chloroplasts are quite evenly distributed on them, 34,5% should be found on the shorter side walls. The results of measurement is presented in fig 6. In the whole range of UV till 380 nm the obtained values are near the theoretical ones for even distribution. From 400 nm till the longest wave length which is capable to bring chloroplasts to a low light arrangement the obtained values are very low when the vibration plane is perpendicular to the short walls and much higher when the light vibrates parallel to them. No significant differences could be found within the spectral regions in question.

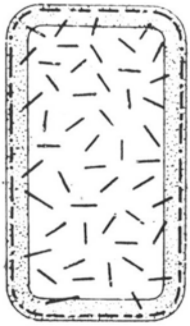


Fig. 7. Postulated orientation of the oscillator responsible for light absorption in the photoreceptor molecules.

DISCUSSION

The chloroplasts have the ability to gather in these places in the cell where light conditions are optimal. Some facts indicate that the photoreceptor active in this phenomenon is localized in the cytoplasm and not in chloroplasts themselves. This was shown by using a narrow beam of light directed on the part of the cell devoided at a given moment of chloroplasts. The irradiated part became the attraction center for the displacing chloroplasts (Fischer-Arnold 1963; Mayer 1964). The peculiar behaviour of chloroplasts after irradiation with polarized light shows that the molecules of the photoreceptor must be dichroic and oriented in the cell structures. A conclusion must be drawn from this experiment that the oscillator responsible for the absorption in blue region of light is situated parallel to the cell surface. When the vibrating plane of light is then parallel to the direction of the oscillator — the absorption of light in the photoreceptor is high and the chloroplasts have the tendency to accumulate in this place in the low light intensity region and to avoid it in the high intensity of illumination. Similar suggestion concerning the orientation of the photoreceptor molecules were drawn from Mayer's (1964) experiments with polarized light on *Selaginella*. Recently Seitz (1966) showed that the photoreceptor responsible for negative phototaxis of chloroplasts, photodinesis and decrease of cytoplasmic viscosity in high light intensity in *Vallisneria* cells is oriented in such a way that the axis of the main absorption of its molecules is parallel to the cell surface. The present results on *Funaria* are in full agreement with the two above mentioned observations.

There arises a question what the orientation of the oscillator of molecules is at the cell surface when looking at it perpendicularly. Some suppositions may be based on the % E — light intensity dependence (Fig 2). When the molecules showing strong dichroism are situated at random on the plane surface, the absorption of such system is two times higher for unpolarized than for linear polarized light. Circular polarized light is absorbed in the same degree as unpolarized one. The behaviour of chloroplasts, when flat arrangement is used as a starting position, shows that there is no difference in their sensitivity to light between not polarized and circular polarized light and that linear polarized light is 1,5—2 x lower in its activity. On the ground of this observation it may be supposed that the direction of the molecules of the photoreceptor oriented parallel to the cell surface shows no or only neglegible orientation of the oscillator of photoreceptor molecules as shown on fig 7.

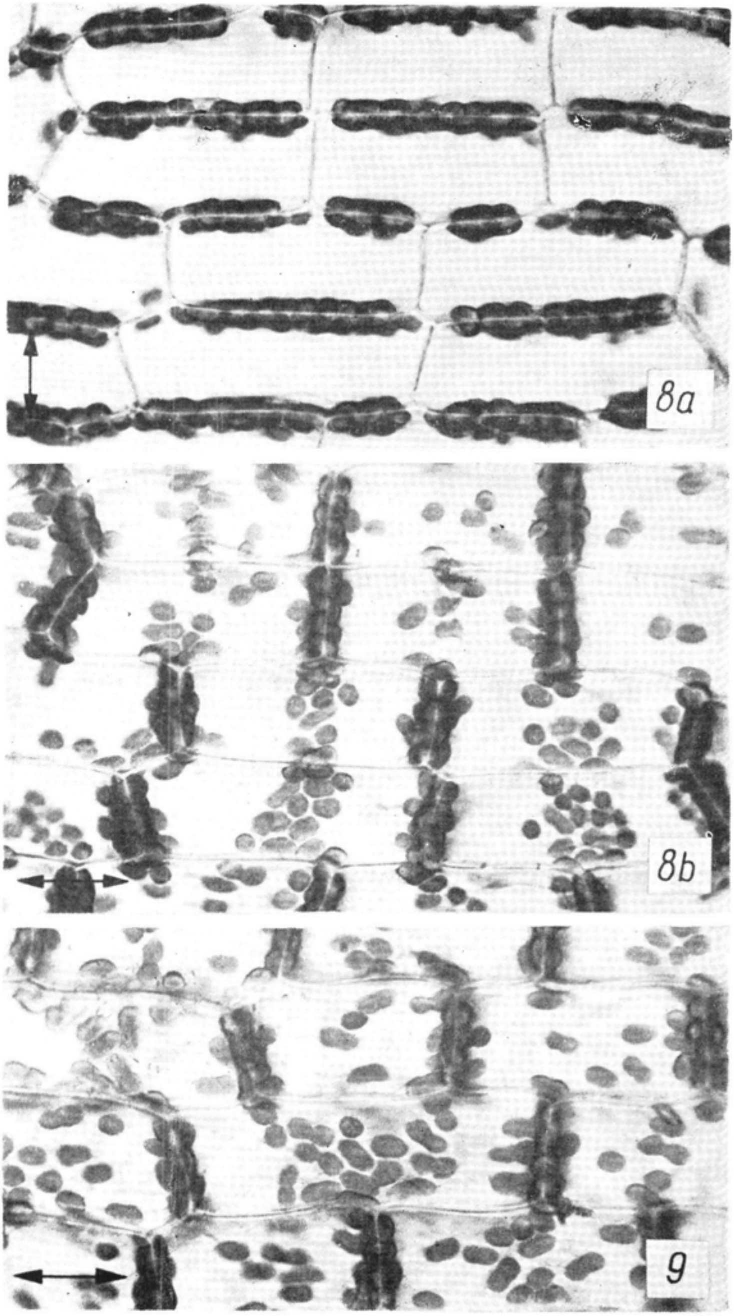
What can be thought about the orientation of the molecules of the photoreceptor on the base of the orientation of the oscillator responsible for absorption? According to the theory, the maximum absorption occures either parallel or perpendicular to the plane of the conjugated bounds of the molecules (Goedheer 1957). Let us assume that riboflavin acts as a photoreceptor. In spite of a rather complicated sterical structure of this compound (Szent-Gyorgyi 1957) the alloxazine ring, which should be responsible for the light absorption because of its conjugated bounds, forms a planar structure. It should be supposed that the orientation of riboflavin is such that its alloxazine ring is lying parallel or perpendicular to the cell surface. In order to accept one from the above mentioned alternatives a detailed knowledge of dichroic properties of the riboflavin molecules is necessary.

Lack of „polarotropic" reaction of chloroplasts in the UV region may be explained in two ways:

1. It can not be excluded that dichroic properties of riboflavin molecules are strongly pronounced for the oscillator of lowest frequency and very weak or no dichroism occurs at the shorter wave of radiation. Some examples of such properties for photosynthetic pigments and rhodamine B are given by Goedheer (1957). In this case it is possible to assume that orientated riboflavin acting as a single photoreceptor may be responsible for the phenomena stated in polarized light.

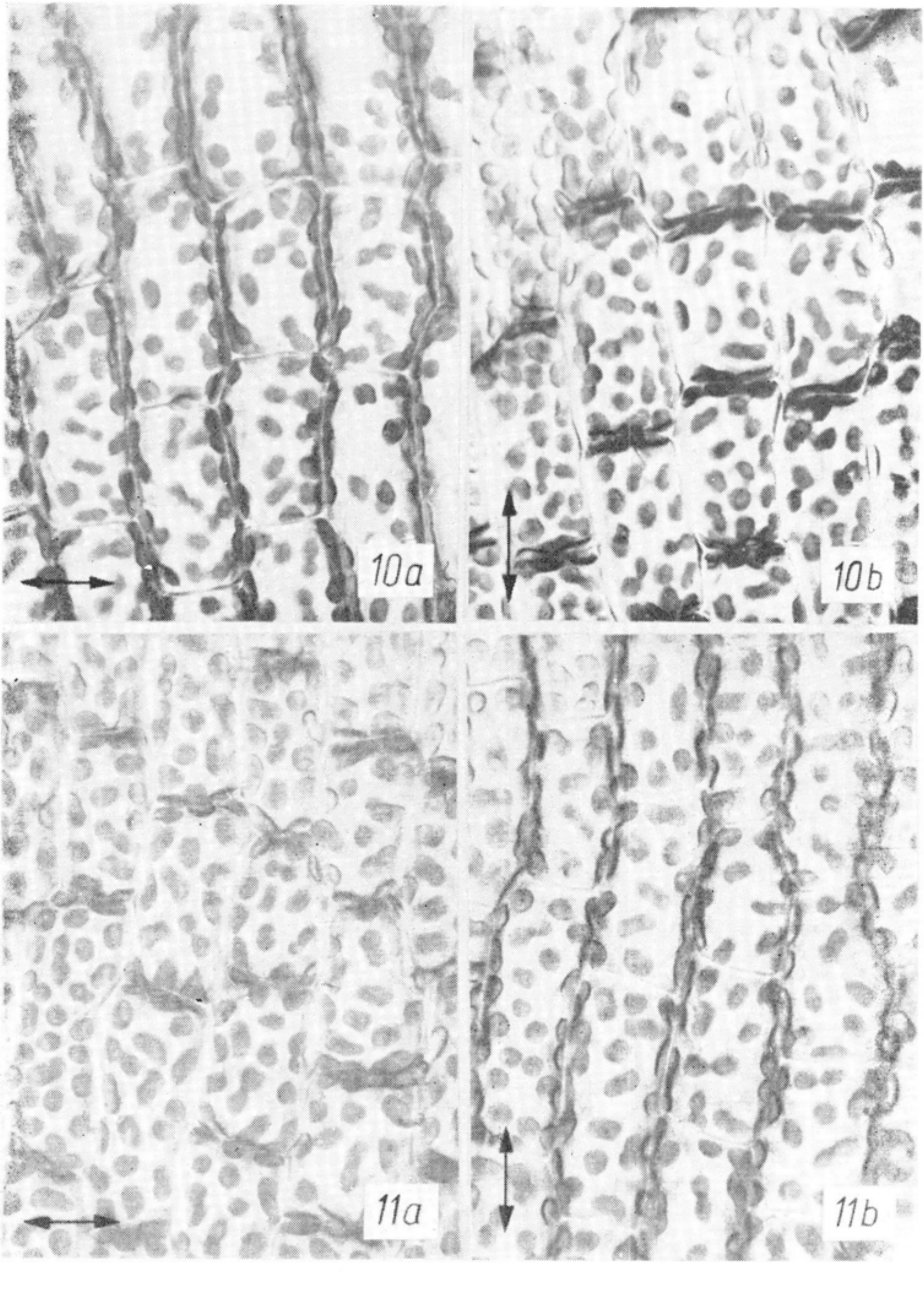
2. If the dichroism of riboflavin molecules is strong in the whole range of absorption it should be postulated that two pigments play the role of a photoreceptor system. The molecules of one of them (riboflavin) are not oriented at all and the orientation of oscillators as shown on fig 7. should be ascribed to another pigment absorbing only in the range above 400 nm.

Plate I



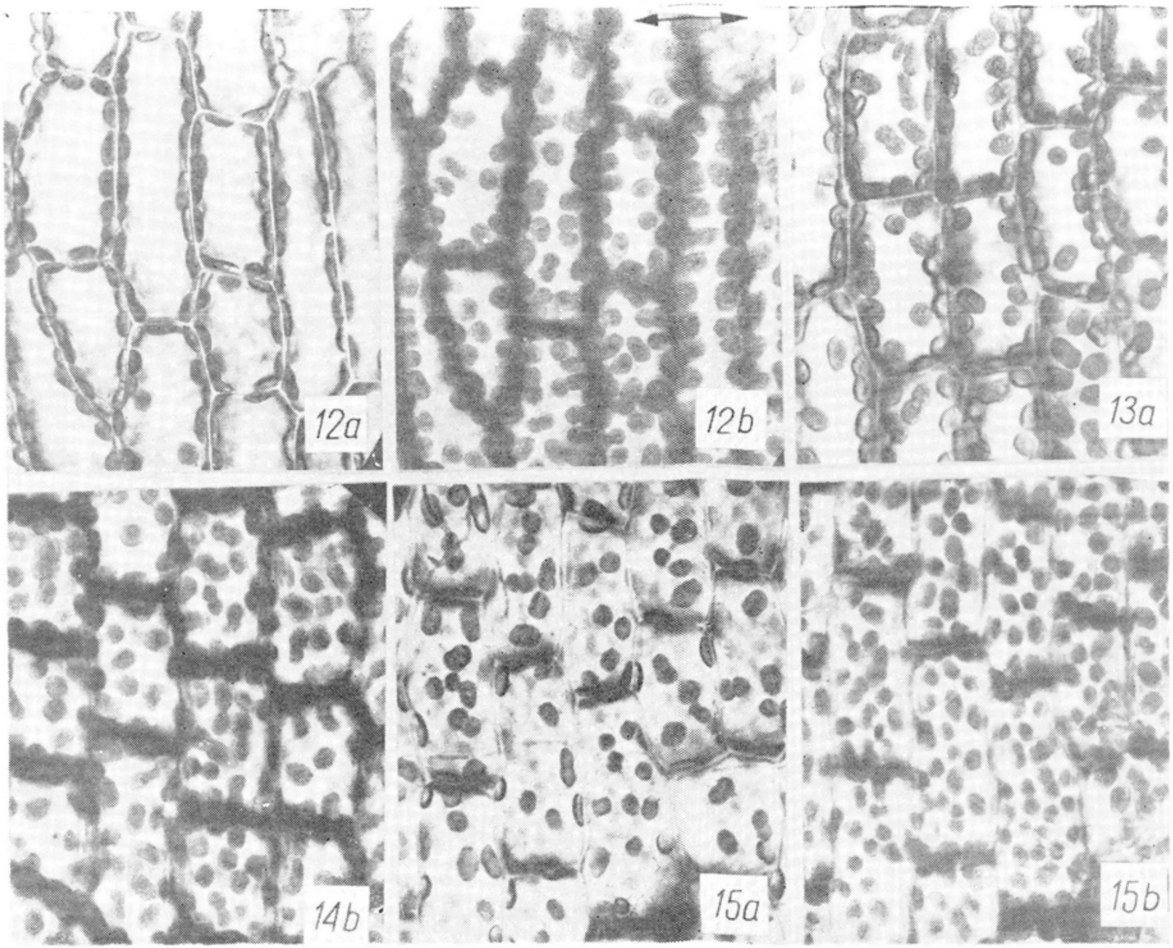
Arrangement of chloroplasts after 1,5 hours irradiation with polarized blue light. The plane of vibration is denoted by arrows. Light intensity 135 000 erg/cm^{2}sec.

Plate II



Arrangement of chloroplasts after 1,5 hours irradiation with polarized blue light.
Fig. 10. Light intensity 20 000 erg/cm^2 sec.; Fig. 11. Light intensity 520 erg/cm^{2}sec.

Plate III



Arrangement of chloroplasts after 1,5 hours irradiation with polarized radiation of the intensities causing half displacements. Radiation vibrate in the plane perpendicular to the longer cell axis. Fig. 12. Wave length 266 nm.; Fig. 13. Wave length 382 nm.; Fig. 14. Wave length 400 nm. Fig. 15. Wave length 523 nm.

a — as focused on the upper (turned away from the light source); *b* — on the lower (turned towards light) wall of thecells

Like in the former case the choice of a right conception is not possible without a detailed study of the dichroic properties of riboflavin molecules.

The study of the kinetics of displacements points to the role of light gradient in the displacements. Not the direction of the light vibration itself, but rather the gradient of light reception within the cell seems to be responsible for the direction of movement. It is clearly visible especially for the chloroplasts which are far from the „attractive" side walls — as in case *b* (fig 5).

The fact that all chloroplasts fail to attain a profile position, when the light vibrates perpendicularly to the longer axis of the cell, seems to be induced not by lack of room for chloroplasts on the shorter side walls, but rather by the fact that some of the chloroplasts are laying in the area lacking any distinct light gradients.

SUMMARY

1. Chloroplasts of *Funaria hygrometrica* show a peculiar arrangements in the cell under illumination with linearly polarized light. They gather themselves on the side walls parallel to the vibration plane of light in low light intensity, and avoid these walls in high light intensity.

2. The phenomenon of polarotropism of chloroplasts can be found in spectral region 400—530nm only. Polarized UV radiation exerts no directing influence.

3. On the base of obtained results speculations were developed concerning orientation of the oscillator responsible for light absorption in the photoreceptor and the orientation of the photoreceptor molecules.

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Własności i lokalizacja fotoreceptora czynnego w przemieszczeniach chloroplastów u Funaria hygrometrica

II. Badania w świetle spolaryzowanym

Streszczenie

1. Światło liniowo spolaryzowane wywiera specyficzny wpływ na ustawienie się chloroplastów w komórce *Funaria hygrometrica*. W słabym świetle chloroplasty gromadzą się na ścianach bocznych równoległych do kierunku drgań światła spolaryzowanego, natomiast w silnym świetle unikają tych ścian.

2. Zjawisko „polarotropizmu" chloroplastów występuje wyłącznie w zakresie spektralnym 400—530 nm. Spolaryzowane promieniowanie nadfioletowe nie wywołuje kierunkowego efektu.

3. Na podstawie uzyskanych danych wysunięto przypuszczenia o kierunkowym ustawieniu w strukturach komórkowych oscylatora odpowiedzialnego za absorbcję światła w drobinach fotoreceptora jak również o ukierunkowaniu samych drobin fotoreceptora czynnego w przemieszczenia chloroplastów.