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The effect of light factor on the development of thallus and generative organs in *Chara vulgaris* L.

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Abstract

During long-term axenic culture of *Chara* continuous illumination (L=24) increases mitotic activity of almost all types of cells. In such conditions only initiation of oogonia is inhibited, leading into a strong predomination of male generative organs. Prolonged darkness (L:D=1:23) exerts a mito-depressing effect. Oogonia and antheridia are especially susceptible to the reduction of the light period. Modifications of the elongating growth in various photoperiods are different in the polyploid regions of the vegetative thallus and in haploid cells of the antheridial filaments. Segments of both axial internodes and lateral pleuridia increase their dimensions at L:D=1:23, whereas at L=24 their growth is significantly inhibited. Different reaction is noted in the cells of antheridial filaments: at L=24 they are about 10 % longer than in the control (L:D=14:10). The duration of the antheridium development, from the stage of unicellular filaments to the moment of antheridium opening, is 1.5 days shorter at L=24 as compared with the control. This shortening includes proportionally both the period of divisions within antheridial filaments and the period of spermatozoid differentiation.

INTRODUCTION

Morphogenetic influence of solar radiation, expressed by changes of plant phenotype, constitutes the effect of an interference into the metabolic systems, growth, and division of cells. So far, studies have not shown any direct effect of light upon the mechanism of functioning of the mitotic apparatus; on the other hand, some modifications of the cell cycle were noted, as well as of the mitotic index and the rate of cellular elongation (for instance, Halaban, 1972; Wada, Furuya, 1972; Furuya, 1977; and ref., Maszewski, 1977).

Structurally and functionally differentiated cells of the vegetative thallus and the generative organs of the alga *Chara vulgaris* constitute

an interesting and relatively simple object for studies on photomorphogenesis. Previous analyses, carried out using a model of synchronously dividing cells of antheridial filaments, showed that continuous illumination shortens the G_2 phase, whereas darkness prolongs this phase, the rate of DNA synthesis remaining unchanged (Maszewski, 1977). Prolonged darkness (3-5 days) results in total inhibition of the mitotic activity in all generations of the antheridial filaments.

The present studies were carried out in order to define the effect of various light conditions upon growth and development of the thallus and generative organs (oogonia and antheridia) in long-term culture of *Chara*.

MATERIALS AND METHODS

Uniform plants of *Chara vulgaris* L. (with respect to the number and size of axial internodes) were grown in axenic conditions on Forsberg's (1965) mineral medium in 150 ml Nessler tubes. Control culture was illuminated for 14 h daily (L:D=14:10) with a set of Flora white glow-lamps, at light intensity of 6.2 $W \cdot m^{-2}$. Some cultures were illuminated continuously (L=24), and some for 1 h only (L:D=1:23).

During the 26-day experiment the plants were moved twice to new media in order to maintain a relatively constant level of mineral components. Observations of plant habit, and growth of thallus and of generative organs, were made every day.

The duration of the antheridium development was defined basing upon an analysis of the period of time elapsing from the formation of 1-celled antheridial filaments (within the distal antheridia of the apical node, at characteristic correlation with calycinal opening of pleuridia — Kwiatkowska, unpubl. data) untill full maturity of spermatozoids, i.e., up to the moment of their liberation after opening of the antheridium.

At the end of culturing period the material was fixed for further microscopic studies in a mixture of glacial acetic acid and ethanol (1:3 v/v) for 1 h, and stained with 2 % orcein and 0.1 % of Fast Green (FCF) solution, according to the Zeiling method.

The length of cells during spermatogenesis was measured with an accuracy of 0.5 μm using an ocular micrometer, on the basis of an analysis of the 64-celled dominating generation of antheridial filaments.

RESULTS

THE EFFECT OF THE LIGHT FACTOR ON DEVELOPMENT OF THE VEGETATIVE THALLUS

Growth and development of morphologically complicated thallus of *Chara* are determined by the frequency of cellular divisions (apical zone)

and cell elongation (mainly of internodes in the subapical zone). Both these processes organize the thallus into a storeyed system of nodes and internodes.

Number of new nodes (dependent on the mitotic activity of apical cell) initiated in continuous illumination slightly exceeds (by 11.1 %) the value characteristic for the control, while the reduction of the illumination period to 1 h per day decreases their number by half (Table 1).

Table 1

The effect of light conditions on the development of the thallus in *Chara vulgaris*

	Control L:D=14:10	Continuous illumination L=24	Reduced period of light L:D=1:23
Increase in number of nodes/14days	2.7±0.1	3.0±0.2	1.4±0.2
Length of internodes (mm)	15.7±0.7	8.3±0.8	21.8±1.0
Increase in thallus length/day (mm)	2.9±0.1	1.7±0.1	1.5±0.3
Length of pleuridium (mm)	12.2±0.9	6.5±0.4	6.1±0.5
Relative increase in number of nodes	100.0%	111.1%	51.8%
Relative increase in number of nodes with rhizoids	100.0%	107.9%	60.5%
Relative increase in number of lateral branches of the thallus	100.0%	158.5%	11.5%

Different effect is noted in the process of elongation growth of internode *Chara* cells (Table 1). The longest cells are observed between the whorls developed at L:D=1:23, and the shortest — at continuous illumination. Growth of the thallus length, which constitutes an effect of both: cell divisions and cell elongation, is most dynamic and harmonious in the control light conditions, when the periods of light and darkness are most close to natural conditions (Table 1).

The effects of various photoperiodic conditions have been also found differentiated, as regards the development of pleuridia (Table 1). At continuous illumination there is an increase in the number of pleuridium cells, with simultaneous reduction of their size as compared to the control. On the other hand, after reduction of the light period, the pleuridia decrease in length which is due to relatively small number of segments. The number of pleuridia growing out of a node is not influenced by light conditions.

Particular segments of the *Chara* thallus are differentiated with respect to age. The youngest whorls, from the apical region constitute

places in which generative organs organize; older ones initiate lateral branches of the thallus and the rhizoids. Relative increase of the number of whorls forming the rhizoids is directly proportional to the number of new thallus segments in all experimental conditions (Table 1).

Continuous illumination stimulates divisions of the apical cells by 11.1 % compared to the control, and results in an increase in the number of whorls forming the rhizoids by 7.9 %. Darkness inhibits formation of new whorls in the apical part of the thallus. It also blocks initiation of new rhizoids. Inhibition of both these processes is respectively 48.2 % and 39.5 %, as compared to the control photoperiod.

Similar correlation was observed with respect to the induction of lateral branches of the thallus. The process is intensified in continuous illumination, and inhibited in darkness, these effects being much more pronounced than in the case of the mitotic activity of apical cells which determines formation of new nodes (Table 1). If an increase in the number of lateral branches in the control is taken as 100%, continuous illumination will stimulate the process by 58.8 %, and the reduction of the light period will inhibit it by almost 90 %.

Table 2

The effect of light conditions on development of the generative organs in *Chara vulgaris*

	Control L:D=14:10	Continuous illumination L=24	Reduced period of light L:D=1:23
Mean number of antheridia of 1 node	13.7±0.6	16.2±0.7	12.7±1.3
Mean number of oogonia of 1 node	13.6±0.9	10.6±0.9	9.3±1.5
Size of antheridium (diameter, μm)	357±5	347±4	degeneration
Cell length (64-celled filaments, μm)	5.3±0.1	5.8±0.1	—
Number of filaments within the antheridium	111.4±4.0	118.9±4.8	degeneration
Number of spermatozoids in the filament	61.3	52.2	—
Number of spermatozoids in the antheridium	6829	6207	—
Number of spermatozoids in the antheridia of 1 node	94921	100547	—
Duration of antheridium development (days)	13.4±0.2	11.9±0.2	degeneration
Duration of the period of cell divisions of antheridial filaments, (h) (after Maszewski, 1977)	160.0	143.0	—
Duration of spermatozoid differentiation (h)	161.6	142.6	—

THE EFFECT OF THE LIGHT FACTOR ON DEVELOPMENT OF THE GENERATIVE ORGANS

Number of generative organs

The number of the antheridia and of oogonia developing in the control conditions is more or less similar, and the quantitative ratio of both organs close to one (1.01, Table 2). Continuous illumination stimulates initiation of the antheridia, increasing their number by 16.5%, and decreasing the number of oogonia by 22.6%. This results in an increase of the antheridia/oogonia ratio to 1.53. Reduction of the light period decreases the number of both organs, this being essentially connected with a reduction of the number of pleuridia segments, but also with a stronger inhibition of oogonia development (the ratio of antheridia/ /oogonia equals 1.36).

Development of the oogonia

In continuous illumination development of the oogonia is normal, and the oogonia do not differ from those developed in the control.

In the L:D=1:23 conditions normal development of female generative organs is disturbed. Processes of degeneration are reflected by gradual but well visible losses of the chlorophyll, leading to total whitening, with simultaneous destruction of the antheridia.

Development of the antheridia and the duration of spermatogenesis

Under the continuous illumination development of the antheridia is similar as in the control, but the period of their development from the stage of unicellular filaments to the stage of antheridium disintegration (and liberation of spermatozoids) is 1.5 days shorter than at the photoperiod of L:D=14:10.

The results of earlier studies (M a s z e w s k i, 1977) suggest that in continuous illumination duration of cell cycles in successive generations of antheridial filaments of *Chara* is shorter due to a reduction of the G_2 phase. Basing upon the above results it can be assumed that the reduction of the period in which antheridium reaches full maturity, induced by continuous illumination, embraces proportionally all mitotic cycles as well as spermatogenesis, i.e., structural transformation of protoplasts into spermatozoids (Table 2, Fig. 1).

Cells in the 64-celled generation, which dominates in the spermatogenesis, become only slightly elongated when cultured in continuous illumination (Table 2). Hence, they show an opposite reaction than the elongation of the thallus internode cells.

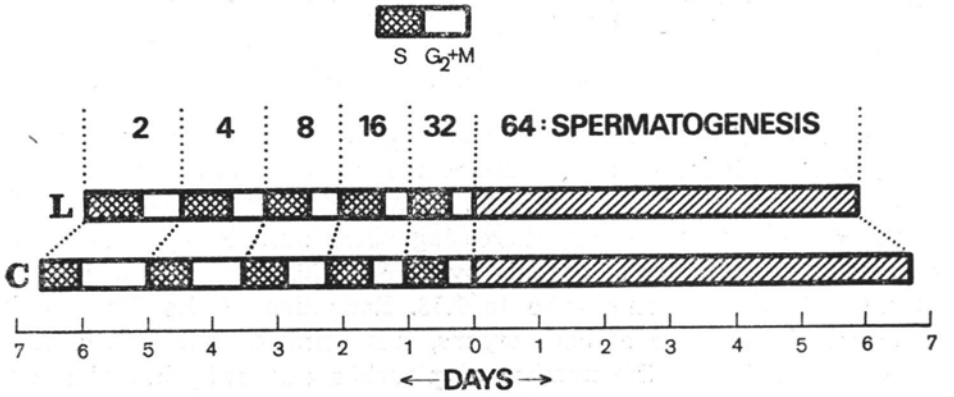

Fig 1. Duration of development of antheridial filaments
C — control (L:D=14:10); L — continuous illumination (L=24); 2, 4, 8, 16, 32, 64 — successive generations of antheridial filaments

Antheridia initiated during prolonged culture in L:D=1:23 conditions show an inhibition of the volume growth after 20 days of slow development, as well as gradually intensified degenerative changes. Microscopic analysis of such antheridia reveals 1-, 2-, or rarely 4-cellular antheridial filaments with totally or partly digested protoplasts. Thus, insufficient illumination causes blocking of the antheridium development during an early stage of the development of antheridial filaments. Under the conditions of the reduced light period the processes of spermatogenesis which bring maturation of the antheridia to an end, occur normally only if they were initiated in control conditions, before experimental changes of the photoperiod.

Biological productivity of the antheridia

In continuous illumination average number of the antheridial filaments, formed as a result of cell divisions of the capitulae, is similar to the control values (Table 2). On the other hand, number of cells in the antheridial filament decreases by about 15 % (Table 2). The results of population analysis (Fig. 2) show that this phenomenon is basically restricted to the fluctuations of the content of filaments containing either more or less than 64 cells. There are no changes in the percentage share of 64-celled filaments, characteristic for the spermatogenesis in *Chara vulgaris* antheridia.

Biological productivity of the antheridia is defined by the number of spermatozoids formed within the filaments (Table 2). Number of spermatozoids developing in the antheridia of the control plants exceeded by about 600 (10 %) number of spermatozoids in the antheridia of *Chara* cultured in continuous illumination. However, when calculation is made

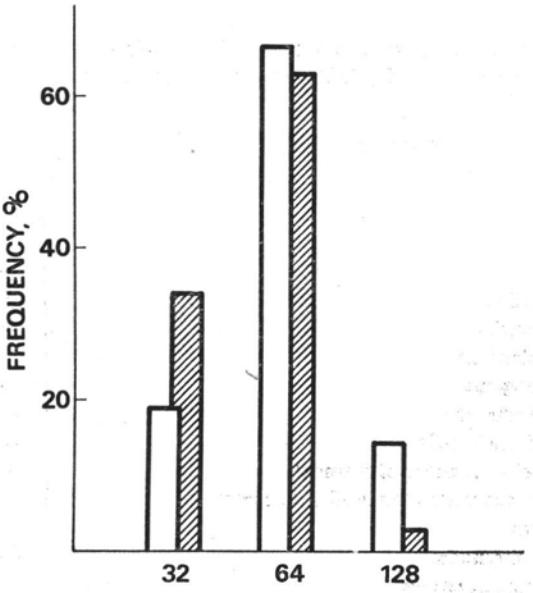

Fig. 2. Frequency distribution of 32-, 64-, and 128-celled antheridial filaments Empty diagrams — control (L:D=14:10), Striped diagrams — continuous illumination (L=24)

per node (higher number of antheridia) or per plant (accelerated formation of new nodes) and all levels of the thallus organization are taken into account, it appears that prolongation of the light period stimulates productivity of spermatozoids by about 17 %.

DISCUSSION

Interference of light factor in the morphogenetic processes of plants takes place through a modification of the two main development factors: frequency of mitotic divisions and elongation of cells.

Prolonged light period stimulates divisions of almost all types of cells, both in haploid regions of the vegetative thallus of *Chara* (apical cells, axial nodes of the plant, pleuridial nodes) and in the generative development of antheridia (reduction of the period of divisions leading to the formation of 64-celled filaments) (Table 3). Similar positive photoreactions were described for many species of algae, fungi, and ferns (for instance, Davis, 1971; Larpent-Gourgaud, Larpent, 1973; Larpent, 1973; Murray, Dixon, 1973, 1975).

Independently of the acceleration of mitotic cycles of apical cells, stimulating effect of continuous illumination is reflected in *Chara* in the process of rhizoid induction and lateral branching of the thallus. This process occurs as a result of mitotic activation of some cells in the ontogenetically older axial nodes of the plant, where the effect of apical

Table 3

Development of the thallus and generative organs in *Chara vulgaris* under continuous illumination (CI) and photoperiod reduced to 1 h/day (RP), in comparison to the control conditions (L:D=14:10)

Process	CI	RP
I. Cell divisions		
1. Thallus apex	+	—
2. Lateral buds	+	—
3. Rhizoid formation	+	—
4. Number of pleuridia	=	=
5. Segments of pleuridia	+	—
6. Initiation of oogonia	—	—
7. Initiation of antheridia	+	—
a) number of shield cells	=	=
b) capitular cells—number of filaments	+	not studied
c) number of cells within antheridial filaments	+	degeneration
II. Elongation growth		
1. Cells of axial internodes	—	+
2. Cells of pleuridia internodes	—	+
3. Thallus	—	—
4. Pleuridia	—	—
5. Cells of antheridial filaments	+	—
III. Spermatogenesis		
1. Duration of spermatogenesis	+	degeneration
2. Productivity of an antheridium	—	degeneration
3. Productivity of antheridia/node	+	degeneration
4. Productivity of antheridia/plant	+	degeneration

+ stimulation; = lack of effects; — inhibition

domination, shown by Libbert and Jahnke (1965), is less pronounced. Hence, it seems that continuous illumination stimulates the rate of cell divisions but decreases the effect of the apical zone. Consequently, both phenomena allow the axillary buds of the thallus to initiate in younger segments laying closer to the apex than in the control material. Intensification of the initiation of lateral thallus branches in continuous illumination can also result from an overproduction of assimilates and increase of their concentration in the plant, as illustrated by the formation of osmiophillic granules within the plastids (Descomps, 1971) and significant increase of dry matter content (Forsberg, 1965). Vegetative growth of the thallus would then take place without use of energetic and nutritive resources from the main axial part of the thallus. Prolonged darkness results in an opposite effect; in view of metabolic deficits it may lead to predomination of the maternal, axial thallus.

Contrary to the relation between light factor and divisions of the thallus cells in *Chara*, which can be expressed by positive correlation

between length of the photoperiod and frequency of mitotic divisions, the effect of light conditions upon elongation growth of cells is much more complex (Table 3). In extreme conditions of continuous illumination and continuous darkness reaction of the generative haploid cells is totally different than the effects noted in the polyploid multinucleate cells of the vegetative thallus. Stimulation of the elongating growth in continuous illumination occurs only in the cells of antheridial filaments, i.e., in the generative zone with specific character of development and differentiation (compare Maszewski, 1977). Long-lasting culture in photoperiod restricted to 1 h/day only (which inhibits development and growth of antheridial filaments) significantly stimulates multinucleate cells of the internodes increasing their length both in the subapical region of the main plant axis, as well as in the pleuridia, resulting in a typical symptom of etiolation.

Oogonia and antheridia are especially susceptible to light factor. Their photomorphogenetic reactions seem to be more complex than in other parts of the thallus. Initiation of the antheridia is stimulated by continuous illumination, and inhibited by prolonged culture in L:D=1:23 conditions. Formation of the oogonia is inhibited when periods of light and darkness deviate from normal proportion occuring in natural conditions. Hence, formation of female generative organs seems to be characterized by very precise requirements with respect to the photoperiod. Unbalanced stimulation of development of the antheridia and oogonia in extreme conditions (significantly differing from the control) suggests some disturbances in the metabolism and/or interaction of endogenous plant hormones, possibly from the group of auxins which was discovered in *Chara* by Libbert and Jahnke (1965), or gibberellins noted by Murakami (1966). These substances play a significant role in the determination of plant sex (for instance Galun, 1959). Their biosynthesis, as well as physiological activity, are largely dependent on light factor (ref. Black, Vlitos, 1972).

Blocking of the antheridia development at L : D= 1 : 23 photoperiod occurs in an early stage of development of the antheridial filaments (1-, 2-, 4-celled stages). Processes of spermatogenesis which take place in the antheridia are normal in case of shortage of the light period only if cellular divisions had ended before the experimental reduction of the photoperiod.

Developmental processes are differently modified by light factor in the region of the vegetative thallus and generative organs. This may be the result of differentiated regulation of gene expression in cells of the syncytial character, with polyploid nuclei, and in specialized haploid cells engaged in the gametogenesis of *Chara* (Moutschen, 1977).

Range of possible modifications of the given features by light factor is different in different cell systems, but always restricted only to

changes of quantitative relations without any impact on phylogenetically stabilized qualitative features of the organism, which determine general structure of the plant and its specialized organs.

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Wpływ czynnika świetlnego na rozwój plechy i organów generatywnych Chara vulgaris L.

Streszczenie

Podczas długotrwałej, aksenicznej hodowli *Chara*, światło ciągłe (L=24) wzmaga aktywność podziałową niemal wszystkich typów komórek; hamowaniu ulega w tych warunkach jedynie inicjacja oogoniów, powodując silną dominację ilościową męskich organów rozrodczych. Długotrwałe dobowe zaciemnienie (L:D=1:23) oddziaływuje mitodepresyjnie. Szczególnie silną wrażliwość na warunki niedoborów światła wykazują oogonia oraz anterydiostany. Modyfikacje wzrostu wydłużeniowego w różnych warunkach fotoperiodu są odmienne w poliploidalnych regionach plechy wegetatywnej oraz haploidalnych komórkach nici spermatogenicznych. Międzywięźla osiowe i segmenty pleurydiów zwiększają swoje rozmiary przy L:D=1:23, a w znacznym stopniu ograniczają swój wzrost przy L=24. Przeciwną reakcję wykazują komórki nici spermatogenicznych; przy L=24 są one o ok. 10% dłuższe niż w kontroli (L:D=14:10). Okres rozwoju plemni od etapu nici 1-komórkowych do chwili rozpadu anterydium trwa przy L=24 o 1,5 doby krócej niż w hodowli kontrolnej. Skrócenie to obejmuje proporcjonalnie okres podziałowy nici spermatogenicznych oraz czas trwania procesu różnicowania spermatozoidów.