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Effect of gibberellic acid on the formation and development of antheridia and oogonia in *Chara vulgaris* L.

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Abstract

The experiments were performed on *Chara vulgaris* thalli cultivated in axenic conditions. It has been found that application of GA_3 at concentrations of 10^{-9}-10^{-4} M has no effect on the numbers of developing antheridia and oogonia. GA_3 stimulates the gemmation of capitular cells thus increasing the number of filaments in antheridia (by 45 % when 10^{-8} M is used). The number of mitotic cycles preceding the differentiation of spermatozoids is not significantly modified by this gibberellin. The number of spermatozoids in antheridia increases by about 20 % when 10^{-8}-10^{-6} M GA_3 concentrations are used. GA_3 shortens the duration of spermatogenesis (by 2.5 days, at 10^{-7} M concentration). This effect is due to a shortening of the mitotic cycles in filaments. On the other hand, the time of spermatozoid differentiation, which in the control material lasts about 5.5 days, is not changed significantly by GA_3. GA_3 hastens the maturation of oogonia and the formation of oospores — both effects are proportional to the GA_3 concentration used.

INTRODUCTION

The formation of male and female reproductive organs in plants occurs under the control of gibberellins. It has repeatedly been found out that a high level of gibberellins is present during the initiation of male organs, and that the formation of female ones occurs when the level of gibberellins is low and that of auxins is high (Mohan Ram, Jaiswal, 1972; Kulikowska et al. 1978; Chailakhyan, 1979 and ref.). A hastening of the formation of antheridia after GA_3 application has also been found in ferns (ref. Miller, 1968).

The present experiments were performed in order to find out the effect of an exogenous gibberellin GA_3 on the initiation, the number

and the time of development of oogonia and antheridia in *Chara vulgaris* L. The effect of this gibberellin on the number of developing spermatozoids in antheridia has also been investigated.

MATERIAL AND METHODS

The studies were performed on *Chara vulgaris* L. cultivated in axenic conditions on mineral F o r s b e r g' s medium (1965) with the use of artificial illumination (L:D=14:10 what resembled the natural photoperiod). Five-nodal apical segments of *Chara* thalli generating reproductive organs, were cultivated for 21 days in Nessler's tubes containing 100 ml of the medium. The following GA_3 concentrations were used: 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. After 8 days the plants were transferred into a fresh regulator containing medium. In each of the GA_3 concentrations 10-12 plants were cultivated. The cultivation was repeated two and in some occasions (with 10^{-7}-10^{-4} M concentrations) three times.

The following observations were performed every day during the experiment: i) the appearance of new nodes on the main axis of the thalli, ii) the opening of antheridia and liberation of spermatozoids, iii) the maturation of oogonia (assessed on the basis of the change in colour from a white-willow green to a brown hue, characteristic for oospores).

After the end of cultivation the material was fixed in an ethanol--acetic acid mixture (3:1 v/v). Isolated antheridia were stained with acetic carmine and then squashed gently on the slides. These preparations served for estimation of the developmental stage, the number of filaments and the number of spermatids within the filaments. The obtained results were analysed statistically with the use of Student's t test at $p=0.05$.

RESULTS

A. EFFECT OF GA_3 ON THE NUMBERS OF ANTHERIDIA AND OOGONIA

In *Chara vulgaris* antheridia and oogonia are usually formed in pairs in the nodes of thallus pleuridia. In the control material the number of antheridia prevailed that of oogonia but the difference was not statistically significant, due to a high differentiation of particular plants (Fig. 1). Neither the number of antheridia nor the number of oogonia was changed significantly at all applied concentrations of GA_3. Only at 10^{-8} M GA_3 concentration (which caused a certain reduction in the number of oogonia and an increase in the number of antheridia) the antheridia/oogonia ratio changed from 1.7 in the control material to 3.0 in the experimental one.

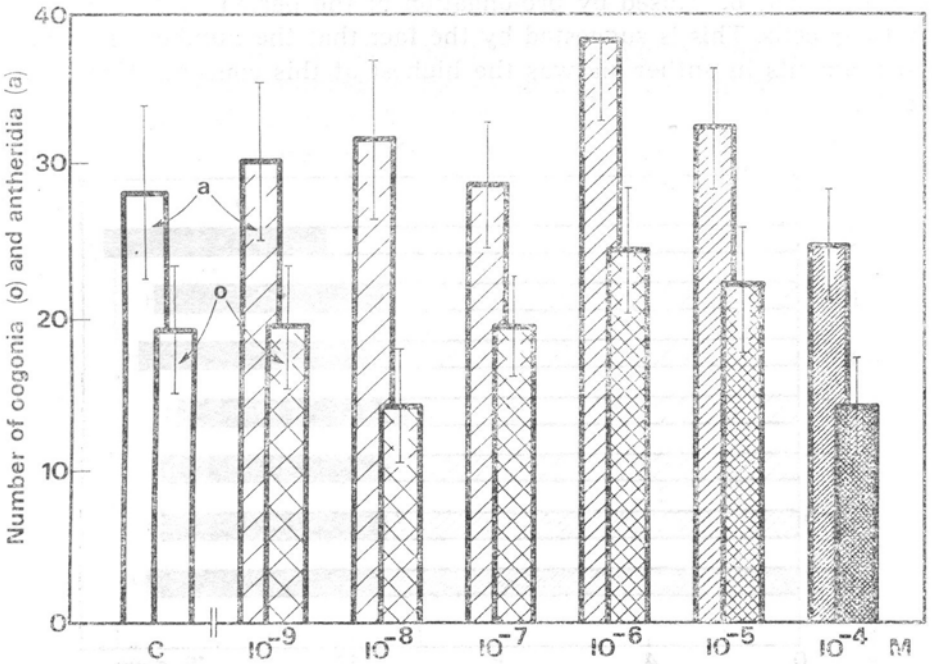

Fig. 1. Number of oogonia (o) and antheridia (a); mean values per plant
C — control, 10^{-9}-10^{-4} — GA_3-treated material

B. EFFECT OF GA_3 ON THE DURATION OF ANTHERIDIA DEVELOPMENT

In *Chara* the development of antheridia is initiated at early stages of the formation of pleuridia and it cannot be observed without a surgical intervention into the apical part of the thallus (Pickett-Heaps, 1967; Ducreux, 1975). Therefore, our observations started from the moment of separation of pleuridia from the apical parts of thalli, what could be observed macroscopically. At this developmental stage shield cells, manubria, capitular cells of the I, II and III order and the initial cells of antheridial filaments are already formed. The latter are formed by unequal divisions (gemmation) of capitular cells. Antheridial filaments develop as a result of synchronous divisions of initial cells leading to the rise of spermatids which differentiate into spermatozoids (Pickett-Heaps, 1967, 1968; Olszewska, Godlewski, 1973).

The mean time of antheridium development (as measured from the initiation of antheridial filaments to the break-up of antheridia) is about 18 days. At all concentrations GA_3 shortens this time. The maximum effect (reduction by about 2.5 days) was observed at 10^{-7} M concentration (Fig. 2). The slight prolongation of the spermatogenesis time observed at 10^{-8} M concentration (as compared to that observed at 10^{-9} and

10^{-7} M) might be caused by prolongation of the period of gemmation of capitular cells. This is suggested by the fact that the number of antheridial filaments in antheridia was the highest at this concentration (comp. Fig. 3).

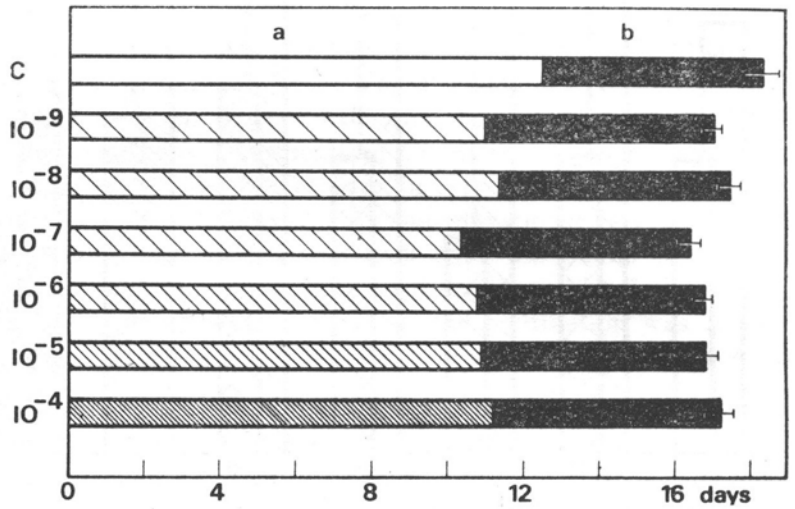

Fig. 2. Duration of the period of mitotic divisions in antheridial filaments (a), and the duration of spermatozoids differentiation (b)
C — control, 10^{-9}-10^{-4} — GA_3-treated material (concentration in M)

The question arised whether the hastening of the antheridium break-up was connected with the shortening of the division period of the antheridial filament cells or was it due to the shortening of the time of differentiation of spermatids into spermatozoids. In order to answer this question the developmental stage of antheridia arising from younger nodes during the experiment was determined. The division time of antheridial filaments was determined on the basis of the time of development of the antheridia which attained the stage of spermatids at the moment of fixation. It lasted 12.5 days in the control material. Thus, about 5.5 days were necessary for the differentiation of spermatozoids from spermatids. Analogous measurements performed on antheridia developing in the presence of GA_3 have shown that the shortening of the total time of antheridium development results exclusively from a shortening of the division period of antheridial filaments (Fig. 2).

C. EFFECT OF GA_3 ON THE NUMBER OF SPERMATOZOIDS DEVELOPING WITHIN THE ANTHERIDIA

The total number of spermatozoids arising in an antheridium depends both on: 1) the mitotic activity of capitular cells, which determines the

number of antheridial filaments, and 2) the number of cellular divisions within a filament.

On the average about 120 filaments are formed in the antheridia of control plants. This number is increased at all concentrations of GA_3. The strongest effect was found at 10^{-8} M concentration where the mean number of filaments was 175 (Fig. 3).

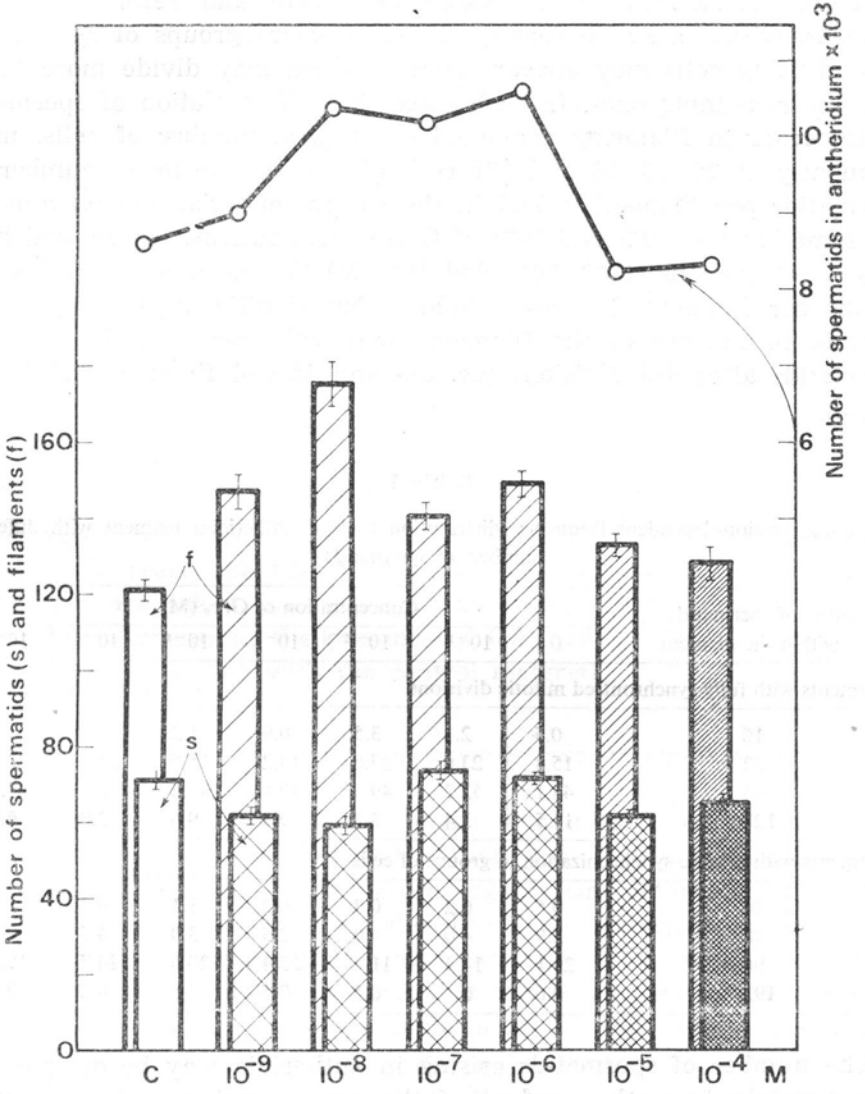

Fig. 3. Mean number of spermatids within a filament (s), mean number of antheridial filaments per antheridium (f), and mean number of spermatids per antheridium

C — control, 10^{-9}-10^{-4} — GA_3-treated material

In control plants a prevealing number of antheridial filaments reaches the stage of spermatids after 6 synchronous divisions i.e. at 64-cell stage (Olszewska, Godlewski, 1973; Olszewska, 1978). In some filaments, however, only 4 or 5 and in some 7 divisions precede the differentiation of spermatozoids (i.e. it occurs in 16- 32- or 128-cell filaments). Moreover, the cell cycles may become desynchronized owing to the plugging of plasmodesmata and, thus, breaking the contact between cells (Kwiatkowska, Maszewski, 1976 and ref.). When this happens, within a single filaments, two or more groups of synchronously dividing cells may appear, some of them may divide more times that the remaining ones. In such cases the differentiation of spermatozoids occurs in filaments composed of atypical number of cells, most frequently of 24, 48, 96 and 192 cells (Table 1). The mean number of spermatids per filament is 71.4 in the control material and remains on the same level at 10^{-7} and 10^{-6} M GA_3 concentrations. At low and high GA_3 concentrations (10^{-9}, 10^{-8} and 10^{-5} M) the mean number of spermatids per filament decreases slightly (but significantly) owing to an increase in number of the filaments with cells reaching the stage of spermatids after 4-5 divisions (i.e. 32- and 16-cell filaments) (Table 1, Fig. 3).

Table 1

GA_3 concentration-dependent frequency distribution (%) of antheridial filament with different numbers of spermatids

Number of spermatids within the filament	Concentration of GA_3 (M)						
	0	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Filaments with fully synchronized mitotic divisions							
16	0.5	2.1	3.5	0.9	1.2	2.2	3.8
32	15.8	23.6	27.6	13.2	15.5	21.4	22.4
64	48.1	52.3	49.3	49.6	48.8	52.8	44.2
128	11.5	3.4	3.3	9.8	9.6	3.0	6.0
Filaments with mitotic synchronization in groups of cells							
24	0.5	0.2	0.3	0.2	1.1	0.2	1.3
48	2.9	4.0	4.0	2.5	3.0	4.5	3.5
96	20.1	14.4	11.4	23.4	20.0	14.7	18.5
192	0.6	0	0.5	0.5	0.8	0.2	0.3

The number of spermatids arising in antheridia may be determined approximately from the product of the mean number of filaments in antheridium and the mean number of spermatids in a filament. In the control material, on the average, 8 600 of spermatozoids arise in one antheridium. In the presence of GA_3 (at concentrations of 10^{-8}, 10^{-7} and 10^{-6} M) this number increases up to 10 200-10 600 (Fig. 3).

D. EFFECT OF GA_3 ON THE ELONGATION RATE OF ANTHERIDIAL FILAMENTS

The measurements of the 64-cell filaments at the stage of spermatids have shown that GA_3 increases the mean length of cells by 2-8%; the effect is proportional to the concentration used (Table 2). At 10^{-6}, 10^{-5} and 10^{-4} M this difference was statistically significant. The total length of the 64-cell filaments developing in the presence of GA_3 at 10^{-4} M concentration increases from 308 μm in the control material to 331 μm

Table 2

Length of spermatids in 64-celled antheridial filaments and the rate of elongation of the filaments in response to various GA_3 concentration

Concentration of GA_3 (M)	Cell length (μm)	Rate of elongation (μm/h)
0	4.81±0.10	0.97
10^{-9}	5.00±0.07	1.16
10^{-8}	5.00±0.07	1.13
10^{-7}	4.90±0.07	1.20
10^{-6}	5.08±0.07	1.20
10^{-5}	5.08±0.07	1.20
10^{-4}	5.17±0.05	1.18

in the experimental one. GA_3 causes also a shortening of the total time necessary to reach the 64-cell stage (comp. Fig. 2). When both these parameters are taken into account the mean elongation rate of the antheridial filaments in the presence of GA_3 (10^{-7}-10^{-4} M) is increased by 23% in comparison with the control material.

E. EFFECT OF GA_3 ON THE DEVELOPMENT OF OOGONIA

The developmental stage of oogonia was determined on the basis of the change in colour after fertilization and transformation into oospores. The percentage of the mature oogonia was determined after the end of the experiment for two nodes: the VI — the separation of which was observed during the experiment, and the V — which developes during the experiment but was initiated a little earlier. In the presence of GA_3 (10^{-4} M) about 10% of oogonia in the VI node were transformed into brown oospores. In the control material a similar number of oospores (15%) appeared only in the V node which arised 5-6 days earlier (comp. Kwiatkowska, Godlewski, 1980). In the VI node, in the presence of GA_3 (10^{-4} M), about 50% of the oogonia were fertilized. A somewhat lesser stimulation was observed when 10^{-5} and 10^{-6} M concentrations were used (Fig. 4).

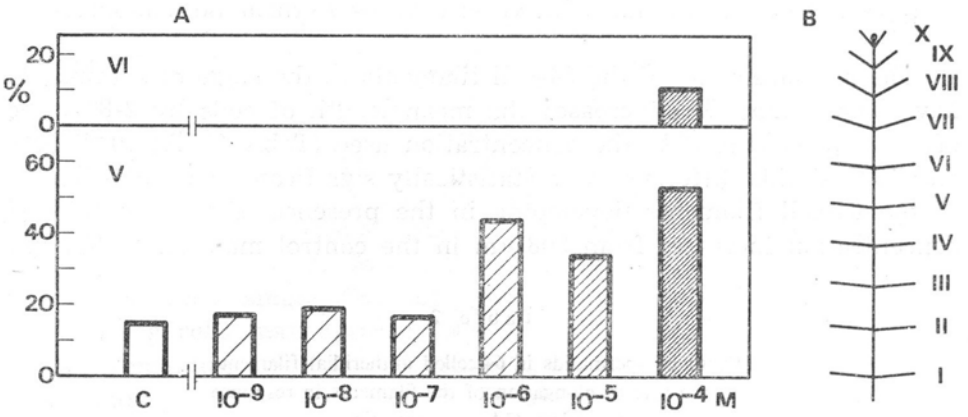

Fig. 4. Relative number (%) of oogonia transformed into oospores
C — control; 10^{-9}-10^{-4} — GA_3-treated material; B — location of analysed nodes within the thallus (for details see the text)

DISCUSSION

The above observations on the effects of the exogenous GA_3 on the formation of the reproductive organs of *Chara vulgaris* have shown no prevailing stimulation of the development of male organs as compared to the female ones. Contrary to the observations on higher plants and on ferns (compare Introduction), high concentration of GA_3 (10^{-5} and 10^{-4} M) more effectively accelerated the maturation of oogonia. The strongest stimulation of spermatogenesis and the highest increase in the number of spermatozoids in antheridia were observed at medium and low concentrations of GA_3 which had no effect on oogonia. The optimum GA_3 concentration increasing the number of antheridial filaments was 10^{-8} M, the strongest acceleration of the development of antheridia was obtained with the use of 10^{-7} M and the highest production of antheridia was observed at 10^{-6} M. A clear-cut dominance of the number of antheridia over that of oogonia was observed only at 10^{-8} M concentration. A similar effect of GA_3 on both male and female reproductive organs has been recently obtained in *in vitro* investigations on *Begonia* (Berghoef, Bruinsma, 1979).

The shortening of the time of the antheridium development by GA_3 occurs through a shortening of the division time of the antheridial filament cells. During this period which comprises successive mitotic cycles a gradual reduction a sizes takes place resulting in attaining the dimensions typical for spermatids (Olszewska, Godlewski, 1972, 1973; Godlewski, Olszewska, 1973). In the dominant population of filaments GA_3 does not reduce the number of these divisions. Earlier investigations concerning the short-lasting influence of GA_3 on the cell cycle of antheridial filament cells of *Chara* (Godlewski, 1977) have

shown that this substance shortens the duration of successive cycles. This effect is particularly strong in younger, 2- and 4-cell stages. In these experiments, analogously as in the present ones where GA_3 was applied for a long time, the total shortening of all cycles amounted to about 2.5 days. It may be assumed then, that the stimulating effect of GA_3 on the development of antheridial filaments is not a transient one. Autoradiographic investigations have shown that in the cell cycle of *Chara* antheridial filaments which are of $S+G_2+M$ type (Olszewska, Godlewski, 1972), GA_3 accelerates both S and G_2 phases exerting a stimulating effect on RNA and protein synthesis (Godlewski, 1977). Similar metabolic effects of GA_3 on RNA synthesis have also been shown in higher plants with the use of biochemical methods. Moreover, it has been found that it is the activity of RNA polymerase I, which is stimulated (Jankowski, Kleczkowski, 1978; Wielgat, Kahl, 1979).

The shortening of the cell cycle duration in antheridial filaments by GA_3 is connected with a stimulation of the rate of cell elongation (comp. Table 2). A similar action typical of GA_3 was observed in young, oospore-grown thalli (Imahori, Iwasa, 1965). On the other hand, the elongation of internodes of the thalli developing reproductive organs is inhibited proportionally to the GA_3 concentration used (Kwiatkowska, Godlewski, 1980).

The increased production of spermatozoids, evoked by a wide range of GA_3 concentrations, is connected with stimulation of the gemmation of capitular cells, what determines the number of filaments in antheridia. The effect of GA_3 on these divisions is opposite to that exerted by IAA (IAA decreases the number of antheridial filaments). On the other hand a decrease in the effectiveness of auxins by TIBA and PCIB leads to an increase in the number of filaments (Godlewski, unpublished) similarly as GA_3 application. Preliminary investigations on the effects of AMO-1618 have shown that a decrease in the level of endogenous gibberellins results in a decrease of spermatozoids production in antheridia, a decrease in the number of filaments and in the number of cell divisions in filaments. AMO-1618 prolongs also the development of oogonia (Godlewski, Kwiatkowska, unpublished). The present data allow us to conclude that gibberellins which are present in the thallus of *Chara* (Murakami, 1966) participate in the formation of reproductive organs in this alga.

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Wpływ kwasu giberelowego na powstawanie i rozwój anterydiostanów i oogoniów Chara vulgaris L.

Streszczenie

W aksenicznej hodowli *Chara vulgaris* L. liczba tworzących się anterydiostanów i oogoniów w obecności GA_3 w stężeniu od 10^{-9} do 10^{-4} M nie ulega istotnej zmianie w stosunku do kontroli. Stwierdzono stymulujący wpływ GA_3 na pączkowanie komórek główkowatych wyrażający się wzrostem liczby nici w plemni (w stężeniu 10^{-8} M o ok. 45%). Liczba cykli mitotycznych poprzedzających różnicowanie spermatozoidów nie jest modyfikowana istotnie przez tę giberelinę. Liczba plemników w anterydiostanach jest zwiększona o ok. 20% w stężeniu 10^{-8}-10^{-6} M. GA_3 skraca czas spermatogenezy (maksymalnie o ok. 2,5 dnia w stężeniu 10^{-7} M) w wyniku skrócenia cykli podziałowych w niciach, natomiast okres różnicowania spermatozoidów trwający ok. 5,5 dnia nie zmienia się w porównaniu z kontrolą. GA_3 przyspiesza dojrzewanie oogoniów i powstawanie oospor wprost proporcjonalnie do stężenia.