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# Studies on the role of gibberellins in the regulation of spermatogenesis in *Chara vulgaris* L.

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## ABSTRACT

Antheridia from isolated nodes of *Chara vulgaris*, developing in the presence of either AMO-1618 or $GA_3$, were studied. AMO-1618 which lowers the level of endogenous gibberellins causes a significant, proportional to the concentration, reduction in: 1) the number of antheridial filaments formed in antheridia, 2) spermatid number within a filament, as a result of eliminating one mitotic division at the first stage of spermatogenesis. Exogenous $GA_3$ at the concentration $10^{-5}$M evokes opposite effect i.e. increase in the number of antheridial filaments and increase in the number of spermatids within filament. Total number of spermatids within an antheridium decreases under the influence of $10^{-4}$M AMO-1618 three times in comparison with the control, whereas it increases twice following $10^{-5}$M $GA_3$ treatment. It has been suggested that the normal course of spermatogenesis requires precisely determined level of endogenous gibberellins.

*Key words: spermatogenesis, $GA_3$, AMO-1618, Chara vulgaris L.*

## INTRODUCTION

Experiments with the use of exogenous $GA_3$ have shown the development of thallus and generative organs of *Chara vulgaris* to be regulated by this hormone (Kwiatkowska and Godlewski 1980, Godlewski and Kwiatkowska 1980). $GA_3$ from $10^{-7}$ to $10^{-4}$M causes, proportionally to the concentration, an increase (up to 400%) in the number of formed oospores as result of their accelerated maturity (Godlewski and Kwiatkowska 1980). Antheridial development is, however, stimulated to a small extent by low concentrations of exogenous $GA_3$ only. $10^{-7}$ M $GA_3$ shortens spermatogene-

sis from 18.5 to 16 days, resulting from the shortened durations of cell cycles at the first stage of spermatogenesis, preceeding the second stage – spermiogenesis. Total number of spermatozoids formed in an antheridium increases slightly (by 20%) as the effect of $GA_3$ treatment, at the concentrations from $10^{-6}$ to $10^{-8}$M (Godlewski and Kwiatkowska 1980).

We have also found that stimulating effect of exogenous $GA_3$ on the development of generative organs of *Chara* is parallel to its inhibitory effect on elongation of multinucleate polyploidal cells of pleuridium internodes (bearing generative organs) as well as internodes of thallus main axis (Kwiatkowska and Godlewski 1980). Increase in the lengths of these cells was achieved, however, by lowering the level of endogenous gibberellins due to AMO-1618 (Kwiatkowska and Godlewski 1980), a gibberellin synthesis inhibitor (Lang 1970). This fact provided the basis for the assumption that in generatively mature *Chara vulgaris* thallus the high level of gibberellin, essential for the development of generative organs, is supraoptimal for the growth of vegetative part of thallus (Kwiatkowska and Godlewski 1980). Moreover, generative organs seem probably to import gibberellins from the thallus.

The aim of the present studies was to check the effect exogenous $GA_3$ and that of an antigibberellin (AMO-1618) on the course of spermatogenesis in *Chara vulgaris* antheridia which developed on isolated thallus nodes at their early developmental stages. These nodes were thus cut off the lying below slightly older nodes with probably the highest gibberellin contents. The latter nodes show, thus, the strongest reduction in elongation due to exogenous $GA_3$ (Kwiatkowska and Godlewski 1980).

## MATERIAL AND METHODS

*Chara vulgaris* L. was collected from the pond localized in Sokolniki village (Łódź district). The plants were cultivated under laboratory conditions (4 Klux, L:D = 16:8, pH 7.4, 22-26°C) in water from the same pond.

From generatively mature plants there were excised: 1) node I located just under the apical bud, the antheridia of which were at the stage of capitular cell divisions and antheridial filament initiations; 2) node II with the antheridia at the stage of initiation and first divisions of antheridial filament cells; 3) node III, counting from the apical bud, the antheridia of which were at the stage of spermiogenesis initiation (Fig. 1). In each experiment, 15-20 isolated nodes were incubated either in $10^{-6}$, $10^{-5}$, and $10^{-4}$M $GA_3$ or $10^{-6}$, $10^{-5}$, and $10^{-4}$M AMO-1618. To maintain the constant level of $GA_3$ and AMO-1618 concentrations in the medium the solutions of these growth regulators were changed every third day. The nodes cultivated without adding the growth regulators were the control.

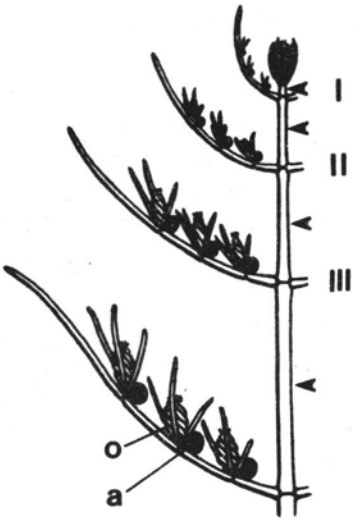


Fig. 1. Scheme of the apical part of *Chara vulgaris* thalli. Arrows indicate the sites of internode cuttings. I, II, and III – node numbers; a – antheridia; o – oogonia

The material was fixed in ethanol-acetic acid mixture (3:1, v/v) after the antheridial filament cells had attained the spermatid stage i.e. the III nodes after 8-, II nodes after 11-, and I nodes after 14-days of culture. Squash preparations from antheridia, stained with orcein and Fast Green were used for quantitative analyses.

## RESULTS

### EFFECT OF AMO-1618 AND $GA_3$ ON NUMBER OF SPERMATIDS IN ANTHERIDIAL FILAMENTS

Under natural conditions, mature antheridia contain about 200 antheridial filaments. Predominant population of these filaments (over 60%) contains 64 spermatids formed as a result of 6 synchronic mitotic divisions of antheridial filament cells during the first stage of spermatogenesis. They are usually accompanied by fewer filaments containing 32 and 128 spermatids (Godlewski and Kwiatkowska 1980). In the antheridia developing on the pleuridia of the nodes isolated at their early developmental stages the number of spermatids formed within particular filaments is reduced (Fig. 2) The highest reduction is in the I node which at the moment of isolation was located directly under the apical bud. After 14-days of culture the antheridia from these nodes contain mature spermatozoids mainly in 16- and 32-celled filaments. In the node II, in which the antheridia attained maturity after 11 days of culture, the spermatids occur in 32- and 64-celled filaments. In the node III from which the antheridia during isolation begin the second stage of spermatogenesis (sper-

miogenesis) the filaments with 64 spermatids predominated, just like under natural conditions.

In the antheridia from the nodes I and II, which were developing in the presence of AMO-1618, the number of short filaments containing 16 and 8 spermatids increased, whereas that of the longer ones – with 32 and 64 spermatids – decreased (Fig. 2). The effect was the stronger the higher

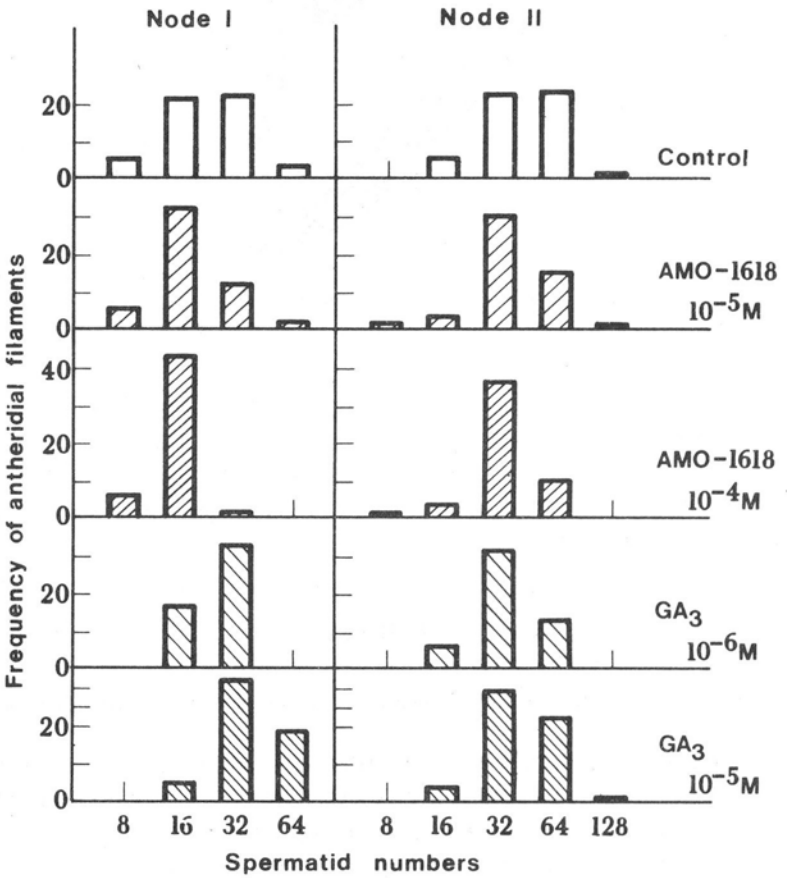


Fig. 2. Effects of $GA_3$ and AMO-1618 on frequency of antheridial filaments containing particular number of spermatids

AMO-1618 concentration was applied and the younger were the antheridia at the beginning of the antigibberellin treatment. The use of exogenous $GA_3$ provided opposite result: the number of longer filaments, with 32 and 64 spermatids increased. Considerable increase of the number of divisions in antheridial filaments however, has been observed in the node I and at $10^{-5}$M $GA_3$ only.

EFFECTS OF AMO-1618 AND $GA_3$ ON THE NUMBER OF ANTHERIDIAL FILAMENTS AND SPERMATOZOIDS IN ANTHERIDIUM

In all the experiments AMO-1618 has distinctly decreased the number of antheridial filaments in the antheridia. The stongest effect i.e. the reduction by 40% in the number of antheridial filaments was observed after the incubation in $10^{-4}$M AMO-1618 (Table 1). However, in the presence of exogenous $GA_3$ the number of antheridial filaments considerably increases, but only in the node I, and at the concentration $10^{-5}$M.

Table 1

Effects of AMO-1618 and $GA_3$ on the average number of spermatids within a filament, on the number of antheridial filaments in an antheridium, and on the number spermatozoids formed within an antheridium

| | Spermatids per filament | | Filaments per antheridium | | Spermatids per antheridium × 1000 | |
|---|---|---|---|---|---|---|
| | number | % | number | % | number | % |
| **Node I** | | | | | | |
| Control | 24.7 | 100.0 | 149 | 100.0 | 3.8 | 100.0 |
| AMO-1618 $10^{-5}$ M | 19.5 | 78.9 | 129 | 86.6 | 2.5 | 66.2 |
| $10^{-4}$ M | 14.2 | 57.5 | 90 | 60.2 | 1.3 | 33.6 |
| $GA_3$ $10^{-6}$ M | 27.3 | 110.5 | 191 | 127.8 | 5.2 | 137.1 |
| $10^{-5}$ M | 43.3 | 175.3 | 194 | 129.8 | 8.4 | 221.1 |
| **Node II** | | | | | | |
| Control | 45.0 | 100.0 | 241 | 100.0 | 10.8 | 100.0 |
| AMO-1618 $10^{-5}$ M | 38.5 | 85.6 | 220 | 91.1 | 8.5 | 78.4 |
| $10^{-4}$ M | 36.0 | 80.0 | 208 | 86.1 | 7.5 | 69.3 |
| $GA_3$ $10^{-6}$ M | 38.7 | 86.0 | 226 | 93.9 | 8.7 | 81.0 |
| $10^{-5}$ M | 44.1 | 98.0 | 270 | 111.8 | 11.9 | 110.0 |

The increase or decrease in the number of antheridial filaments in the antheridium as well as the number of spermatids within a filament due to $GA_3$ or AMO-1618 determines the changes in "productivity" of the antheridia i.e. the total number of spermatozoids formed. At the most effective concentrations, in the antheridia from the nodes I, AMO-1618 reduces spermatid number in the antheridium by 67%, whereas exogenous $GA_3$ causes an increase in their number by about 121% (Table 1). The number of spermatids formed in $10^{-5}$M $GA_3$ treated antheridia is nearly 6 times higher than that in antheridia treated with $10^{-4}$M AMO-1618.

## DISCUSSION

The received results completely confirm the previous conclusion (Godlewski and Kwiatkowska 1980) that gibberellins found in *Chara* thallus by Murakami (1966) have the essential function in regulating spermatogenesis in this alga. Their effects appear distinctly only at the early stages of spermatogenesis: 1) during the formation of the antheridial filament initial cells due to budding of capitular cells; 2) during the divisions of antheridial filament cells as a consequence of which spermatids arise and the preliminary stage of morphogenesis – gradual reduction in cell sizes – occurs. The process of spermatid differentiation, i.e. the period of spermiogenesis, is not sensitive to exogenous $GA_3$ (Godlewski and Kwiatkowska 1980).

The present experimental studies were carried out under the conditions in which level of gibberellins in antheridia can be expected to be lowered as a result of isolating young nodes from more mature part of thallus, rich in gibberellins (Kwiatkowska and Godlewski 1980). It may account for, as we think, the several-fold stronger effect of the increase in spermatozoid number in the antheridium due to the optimal $GA_3$ concentration than that in the former studies during which the whole apical part of thallus was treated with $GA_3$. Previously the most intense stimulation of the studied processes was due to $GA_3$ treatment at the concentrations $10^{-7}$ and $10^{-8}$M. In the present studies the optimal $GA_3$ concentration was $10^{-5}$M, which may also be explained by the lower level of endogenous gibberellins. Thus the regulation of spermatogenesis seems to depend on the precisely determined gibberellin content.

The results of the studies using AMO-1618 provide the essential argument confirming the assumption of significant roles of gibberellins in regulating the first stage of spermatogenesis. This antigibberellin was found to evoke opposite effect to that of exogenous $GA_3$ i.e. 1) reduction in the number of antheridial filaments developing on the antheridia as a result of the diminished budding activity of capitular cells, and 2) decrease in the number of divisions of antheridial filament cells at first stage of spermatogenesis.

The reduction in spermatid number in antheridial filaments due to AMO-1618 is closely related to the reduction in filament lengths as in all the experiments the sizes of particular spermatids are very similar. The increase in the number of spermatids in a filament, due to exogenous $GA_3$ is, however, correlated with the increase in filament lengths. The acceleration of the filament elongation rate as the effect of $GA_3$ is associated with the shortening of successive cell cycles (Godlewski 1977) as well the reduction in the total duration of the first stage of spermatogenesis (Godlewski and Kwiatkowska 1980). The autoradiographic studies (Godlewski 1977) have shown that the influence of $GA_3$ on the development of antheridial filaments is exerted,

among other factors, as a result of the increase in the activity of transcription and translation, both during S and $G_2$ phases of antheridial filament cell cycle (type $S+G_2+M+C$) (Olszewska and Godlewski 1972).

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REFERENCES

Godlewski M., 1977. The role of plant growth substances in the regulation of the cell cycle in antheridial filaments of *Chara vulgaris* L. Effect of gibberellic acid on some processes in the course of the cell cycle. Acta Soc. Bot. Pol. 46: 317-329.

Godlewski M., Kwiatkowska M., 1980. Effect of gibberellic acid on the formation and development of vegetaive systems in generatively matured thalli of *Chara vulgaris* L. Acta 459-469.

Kwiatkowska M., Godlewski M., 1980. Effects of gibberellic acid and AMO-1618 on the development of vegetative systems in generatively matured thalli of *Chara vulgaris* L. Acta Soc. Bot. Pol. 49: 445-458.

Lang A., 1970. Gibberellins: structure and metabolism. Ann. Rev. Plant. Physiol. 21: 537-570.

Murakami Y., 1966. Bioassay of gibberellins using rice endosperm and some problems of its application. Bot. Mag. (Tokyo) 79: 315-327.

Olszewska M. J., Godlewski M., 1972. An autoradiographic study of the synthesis of nucleic acids and protein during the cell cycle of synchronously dividing antheridial filaments in *Chara vulgaris* L. Folia Histochem. Cytochem. 10: 245-256.

## *Badania roli giberelin w regulacji procesu spermatogenezy u Chara vulgaris L.*

Streszczenie

Badano anterydiostany izolowanych węzłów plechy *Chara vulgaris* rozwijających się w obecności $GA_3$ lub AMO-1618. Wykazano, że AMO-1618, które obniża poziom endogennych giberelin, powoduje znaczną, proporcjonalną do stężenia redukcję liczby tworzonych w anterydiach nici spermatogenicznych oraz redukcję liczby spermatyd w nici, w wyniku eliminacji jednego podziału mitotycznego w pierwszym etapie spermatogenezy. Egzogenny $GA_3$ w stężeniu $10^{-5}$M wywołuje efekt przeciwny, tj. zwiększa liczbę spermatyd w nici. Całkowita liczba spermatyd w anterydium, w porównaniu z kontrolą, zmniejsza się trzykrotnie pod wpływem AMO-1618 stosowanym w stężeniu $10^{-4}$M i zwiększa się dwukrotnie w obecności $10^{-5}$M $GA_3$. Przeprowadzone badania wskazują, że proces spermatogenezy wymaga ściśle określonego poziomu endogennych giberelin.