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Effects of gibberellic acid and AMO-1618 on the development of vegetative systems in generatively matured thalli of *Chara vulgaris* L.

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Abstract

Effects of GA_3 (10^{-11}-10^{-4} M) and AMO-1618 (10^{-6}-10^{-4} M) on the development of generatively matured thalli of *Chara vulgaris* were investigated during 21-day culture of plants in axenic conditions. It has been found that in the main bud the divisions of apical cells of the thalli are not stimulated by GA_3, whereas in the lateral buds the cell divisions are stimulated by higher GA_3 concentrations. Subsequent mitotic activity of the apical cells in the branches of the main axis is not stimulated by GA_3, whereas the lateral buds of these branches are activated. The development of rhizoids in younger nodes is accelerated by high GA_3 concentrations. The elongation of the polynuclear, internodal cells of the main axis and that of pleuridia are inhibited proportionally to the GA_3 concentration. AMO-1618 stimulates the development of new nodes, elongation of internodes and delays the activation of lateral buds as well as the formation of rhizoids. These results suggest that the GA_3-induced inhibition of elongation of the thalli and diminution of the apical domination is connected with a high level of endogenous gibberellins in the generatively matured thallus.

INTRODUCTION

The stimulation of elongation growth in the stalks of higher plants is one of the most characteristic physiological effects of gibberellins (ref. Lang, 1970). Also, in a variety of algal species the growth rate and cell divisions are activated by GA_3 (Conrad et al., 1959; Jennings, Mc Comb, 1967; Ousheva et al., 1968; Bralczyk et al., 1978).

The investigations performed by Imahori and Iwasa (1965) on the vegetative system of thallus cells in *Chara* have shown that in young plants GA_3 does not stimulate the elongation of protonema but

increases the growth rate of internodes and that of pleuridia. Similar effects were obtained by Starling et al. (1974) in *Nitella hookeri*. A stimulation of elongation has also been observed in the case of antheridial filaments of *Chara vulgaris* (Godlewski, Kwiatkowska, 1980).

The present investigations with the use of GA_3 and AMO-1618 (the latter being an inhibitor of the synthesis of gibberellins) were performed in order to find out whether gibberellins participate in the control of division and elongation of different types of cells in the vegetative system of generatively matured *Chara* thalli.

MATERIAL AND METHODS

The experiments were performed on *Chara vulgaris* L. thalli cultivated in axenic conditions in Forsberg's medium (1965) under artificial light conditions imitating the natural photoperiod (L:D=14:10). The experimental culture was run for twenty one days in Nessler's tubes. Five-nodal apical parts of *Chara* thalli developing reproductive organs were put into 100 ml of medium containing GA_3 at the following concentrations: 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. Eight days later the plants were transferred into fresh, regulator containing medium. In each of the GA_3 concentration as well as in the control medium 10-12 plants were cultivated. The effects of GA_3 at concentra-

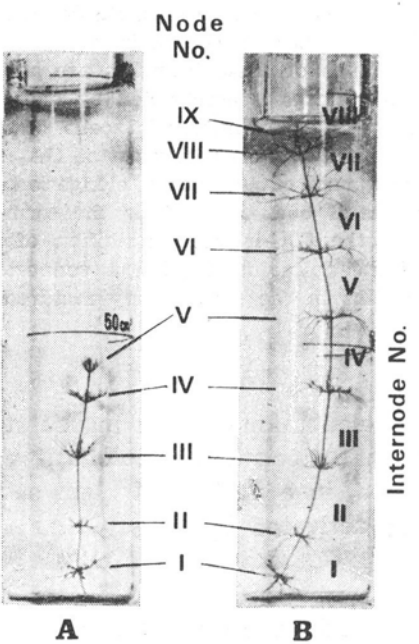

Fig. 1. Apical fragment of *Chara vulgaris* thallus
A — at the start of the experiment, B — after 21 days of cultivation

tions of 10^{-9}-10^{-4} M were tested in three independent cultures. A similar procedure was applied for AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methylchloride), at concentrations: 10^{-6}, 10^{-5} and 10^{-4} M.

The appearance of new nodes on the main axis was carefully observed every day during the experiment. Every second day the plants were photographed as to record the rate of elongation of the main axis internodes and that of pleuridia (Fig. 1). After the end of experiment the number and the length of branches as well as the number of nodes which had developed rhizoids were determined. Some additional observations on the development of branches were performed on isolated nodes with the use of GA_3 at concentrations 10^{-5} and 10^{-4} M. The obtained results were analysed statistically and the significance of differences was evaluated using Student's t test at $p=0.05$.

RESULTS

A. EFFECTS OF GA_3 AND AMO-1618 ON THE MAIN AXIS GROWTH RATE

At none of the GA_3 concentrations the elongation rate of the main axis was stimulated. At concentrations of 10^{-9} to 10^{-4} M the growth was inhibited and the effect was positively correlated with the concentration of the regulator (Fig. 2). This inhibition was stronger in the first phase of cultivation (up to 8-th day). After a longer time the inhibitory effect decreases, particularly at higher concentrations of GA_3 (10^{-4} M). The GA_3 induced decrease in the rate of thallus elongation was not due to a decrease in the mitotic activity of apical cells. At 10^{-7} M GA_3 concentration the time after which the first node separated from the apical part of the thallus was slightly but significantly shorter in comparison with the control material (Table 1). In the next period of cultivation a transient decrease in the appearance of new nodes was

Table 1

Effect of GA_3 on the time elapsing till the separation of the first and second node from the apical bud and the number of nodes formed during 21 days incubation

Concentration of GA_3 (M)	Time (days)		Number of nodes
	first node	second node	
0	5.65±0.14*	3.95±0.15	3.37±0.17
10^{-9}	5.83±0.14	4.13±0.26	3.36±0.14
10^{-8}	5.79±0.19	4.77±0.42	3.38±0.20
10^{-7}	5.23±0.14	4.60±0.23	3.31±0.19
10^{-6}	5.75±0.33	4.58±0.32	3.11±0.18
10^{-5}	6.00±0.20	4.58±0.26	3.18±0.16
10^{-4}	6.00±0.27	4.27±0.28	3.87±0.15

* ±SE

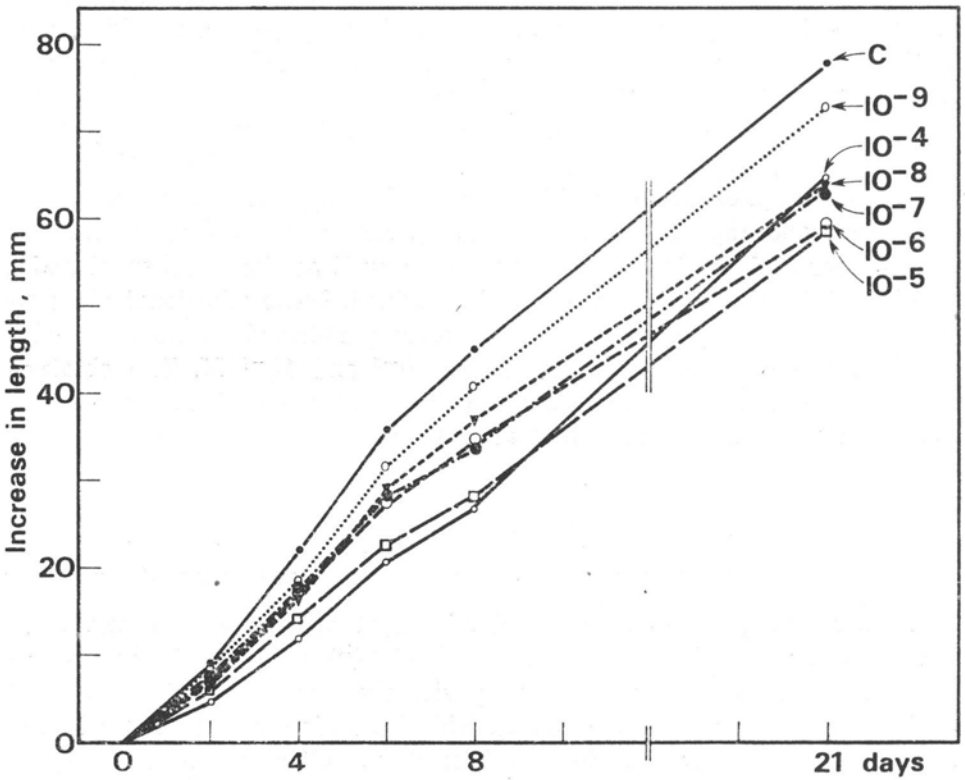

Fig. 2. Increase in length of *Chara* thallus
C — control, 10^{-9}-10^{-4} — GA_3-treated material (concentration in M)

observed. In the last phase of the experiment the facilitation of the development of nodes by GA_3 and their total number were similar to that in the control material (Table 1). There were no differences between the control and GA_3 treated plants in the numbers of cells in nodes as well in the total number of nodes in thalli.

After the decrease in the level of endogenous gibberellins by AMO-1618 (K u o, P h a r i s, 1975) a clear acceleration of the formation of new nodes was observed (Table 2). This effect, however, appeared in the second phase of the experiment, i.e. after 11 days of cultivation.

The GA_3 induced decrease in length of the *Chara* thallus is due to a reduction in elongation rate of the main axis internodes (Fig. 3). It occurs in the oldest internodes (I, II and III) which attained their final stage of development at the start of the experiment as well as in those

Table 2

Effect of AMO-1618 on the growth and development of *Chara* thallus*

	Concentration of AMO-1618 (M)			
	0	10^{-6}	10^{-5}	10^{-4}
A. Elongation of thallus				
Increase in number of nodes	2.1±0.1**	2.2±0.1	2.3±0.1	2.8±0.2
Length of internodes (mm) VII	1.0±0.1	0.9±0.2	1.1±0.1	2.6±0.4
VI	2.8±0.2	2.9±0.2	3.0±0.2	4.9±0.6
Length of pleuridia (mm)				
node VII	1.4±0.2	1.7±0.1	1.8±0.1	2.8±0.3
node VI	3.1±0.4	3.0±0.3	3.5±0.3	3.8±0.3
B. Lateral branches				
Number of branches	3.1±0.4	3.1±0.2	2.6±0.3	3.6±0.3
Number of nodes within the branch	1.2±0.2	1.2±0.2	1.1±0.1	1.7±0.2
Distance from the apex (mm)	35.8±2.9	32.7±3.2	41.6±2.5	42.9±2.7
Number of nodes from the apex	4.2±0.3	4.0±0.2	4.4±0.3	4.8±0.2
C. Rhizoids				
Distance from the apex (mm)	12.9±1.2	13.9±2.1	15.2±1.8	21.8±3.4
Number of nodes from the apex	2.3±0.2	2.4±0.2	2.8±0.2	3.4±0.3

* The experiment with AMO-1618 was performed in other time than that with GA_3; as compared with the latter material, plants incubated in AMO-1618 showed less intense growth.

** ±SE

which were initiated and developed during the experiment (IV, V, VI and VII). GA_3 reduces the length of pleuridia by shortening their internodes. This effect is proportional to the concentration of GA_3 used (Fig. 4). In successive nodes developing during the experiment the GA_3 effect on pleuridia was similar.

Contrary to GA_3, AMO-1618 stimulates the elongation rate of internodal cells both in the main axis and in pleuridia. This effect was pronounced mostly in the second phase of the experiment (after 11 days) in the youngest internodes and it was the strongest at 10^{-4} M AMO-1618 concentration (Table 2 A).

B. EFFECT OF GA_3 AND AMO-1618 ON THE APICAL DOMINATION OF THALLI

In *Chara* development of the main axis ramifications from the buds arising in nodes is initiated at a certain distance from the thallus apex. The distance is the longer the higher is the elongation rate of the main axis. In the control material the distance is about 104 mm and encomposes 6-8 nodes. At higher GA_3 concentrations, which inhibit the elongation of internodes, the activation of the axis buds in younger nodes, lying close to the apical cell (64 mm, 4 to 6 nodes — Table 3) is accelerated. In the control material, in nodes of similar age, lateral

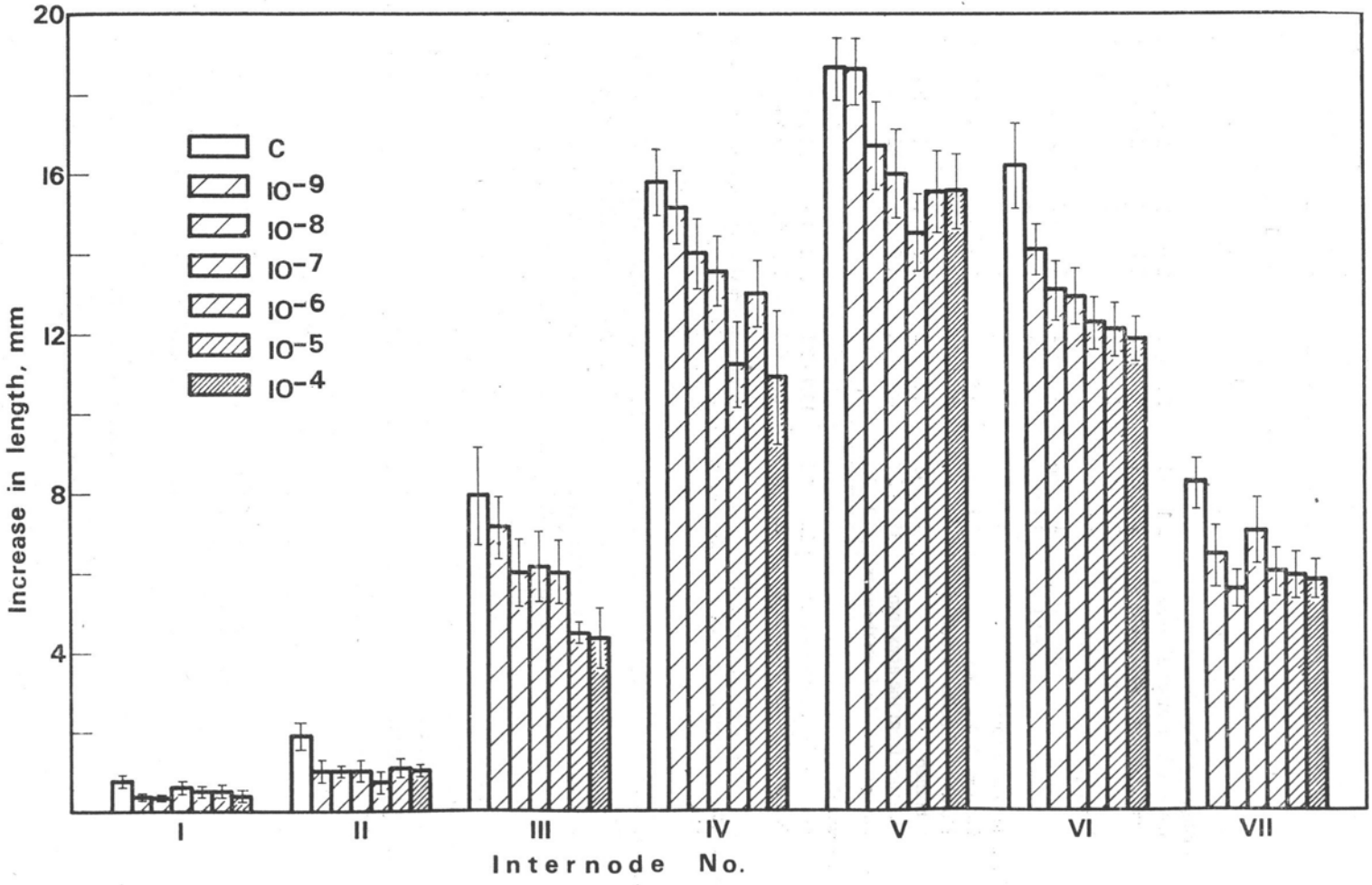

Fig. 3. Increase in length of axial internodes of *Chara* thallus
C — control, 10^{-9}-10^{-4} — GA_3-treated material (concentration in M)

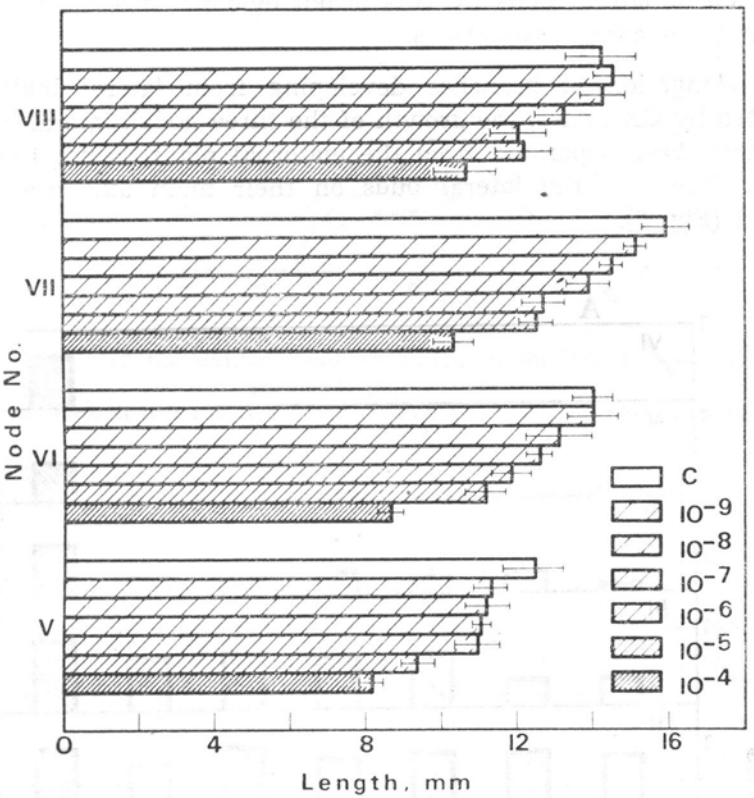

Fig. 4. Length of pleuridia in nodes developed and formed during the experiment C — control, 10^{-9}-10^{-4} — GA_3-treated material (concentration in M)

Table 3

Location of the first activated axillary bud in relation to the position of the thallus apex in control (0) and GA_3-treated plants

Concentration of GA_3 (M)	Distance from the thallus apex	
	Number of nodes	mm
0	6.5±0.3*	104.5±5.8
10^{-9}	5.9±0.2	105.9±6.5
10^{-8}	5.8±0.2	85.0±5.3
10^{-7}	5.8±0.2	89.9±3.7
10^{-6}	5.3±0.4	76.2±6.0
10^{-5}	5.6±0.3	83.2±3.3
10^{-4}	4.7±0.3	63.5±4.3

* ±SE

ramifications may be observed on the IV and V nodes in some plants only. In the GA_3 treated material (10^{-4} M), in majority of plants, these branches are formed on the VI node. In older nodes (I and II) the

stimulation of lateral buds by GA_3 is not evident since they are in an active state in control conditions.

The elongation of branches developing from lateral buds is not stimulated by GA_3. Also, the growth of the three-nodal, lateral branches, which have been separated from the maternal plant, is not accelerated by GA_3. However, the lateral buds on their main axis are strongly activated (Fig. 6).

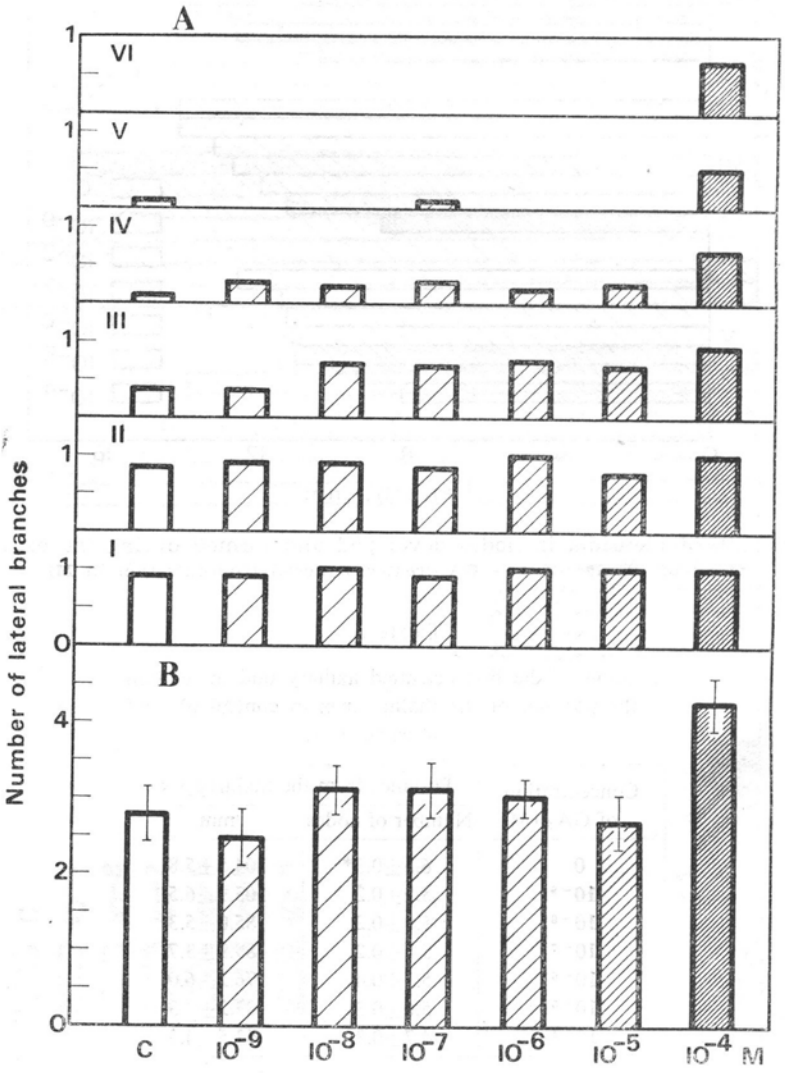

Fig. 5. Number of lateral branches sprouting off the main axis
A — in consecutive nodes, B — mean value per plant, C — control, 10^{-9}-10^{-4} — GA_3-treated material

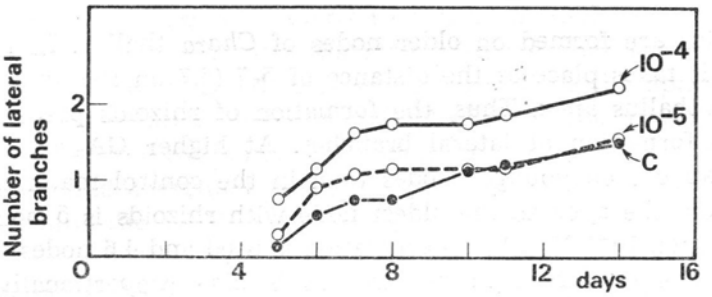

Fig. 6. Dynamics of the axillary bud activation in the lateral branches cut off the main thallus axis
C — control, 10^{-5} and 10^{-4} — GA_3-treated material (concentration in M)

AMO-1618 delays the activation of axial buds on younger nodes but accelerates the growth of the branches developing on these nodes stimulating in this way the formation of new nodes (Table 2B).

Separation of nodes from thallus axis eliminates the apical domination and leads to an activation of the development of axial buds (Ducreux, 1974, 1975). In such an experimental set, GA_3 increases the number of branches developing from a node (Table 4) and stimulates the axial buds of these branches. The number of nodes and the length of the first branch is reduced by GA_3, whereas the growth rate of the subsequently formed branch is accelerated (Table 5). Thus, GA_3 reduces the domination of the oldest lateral branch.

Table 4

Effect of GA_3 (10^{-5}M) on the number of lateral branches developed from isolated nodes

Node No.		II	III	IV
Number of primary branches	Control	1.8	1.0	0.1
	GA_3	2.7	2.3	0.2
Number of secondary branches	Control	0.4	0.2	0
	GA_3	0.6	0.4	0.1

Table 5

Effect of GA_3 on the development of lateral branches developed from isolated nodes after 21 days of incubation

Concentration of GA_3 (M)	Number of nodes in the branch		Length of the branch (mm)	
	First branch	Second branch	First branch	Second branch
0	4.4	1.5	29.3	7.7
10^{-5}	3.7	1.6	25.0	5.9
10^{-4}	3.6	2.3	26.7	13.0

C. EFFECTS OF GA_3 AND AMO-1618 ON RHIZOID FORMATION

Rhizoids are formed on older nodes of *Chara* thallus. In the control material it takes place at the distance of 5-7 (5.7 on the average) nodes from the thallus apex. Thus, the formation of rhizoids precedes by one node the formation of lateral branches. At higher GA_3 concentrations rhizoids appear on younger nodes than in the control material. The distance from the apex to the oldest node with rhizoids is 5 nodes on the average when 10^{-5} M GA_3 concentration is used and 4.6 nodes at 10^{-4} M. The distance expressed in centimeters is also proportionally reduced (Fig. 7).

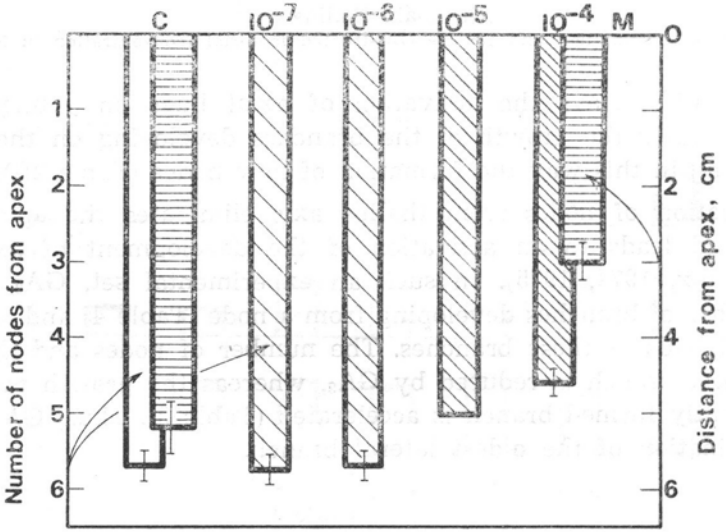

Fig. 7. Location of the first node forming rhizoids, in relation to the thallus apex
C — control, 10^{-7}-10^{-4} — GA_3-treated material (concentration in M)

AMO-1618 exerts an opposite effect to that produced by GA_3, i.e. rhizoids are formed on nodes more distant from the apex (more then one node further on the average, Table 2).

DISCUSSION

Two types of cells have been distinguished in the vegetative system of *Chara* thallus: i) mitotically dividing apical cells and the nodal cells of the main axis and of pleuridia with the DNA level 1C—2C; ii) internodal cells which do not divide but increase in length intensely (Pickett-Heaps, 1967; Shen, 1967a; Ducreux, 1975 and ref.). During the elongation of internodal cells DNA is synthetized intensely (Moutschen, 1977) leading to the formation of large polyploid nuclei (Shen, 1967a). As many authors assume, these nuclei divide amitoti-

cally (Shen, 1967b and ref.; Roberts, Chen, 1975; Moutschen, 1977). Cytophotometric investigations have shown that these amitoses, which occur in a complicated, precise way, result in the appearance of nuclei containing identical amounts of DNA (Shen, 1967a). As the internodes become longer the number of nuclei increases up to about 2 000. At the same time a gradual reduction in the DNA content in a single nucleus reaching the 1C-2C level in the oldest internodes (Shen, 1967a and b).

The results of our investigations have shown that the response to GA_3 is different in these two vegetative systems of generatively matured *Chara* thalli. The polynuclear, internodal cells of the main axis and those of pleuridia show consistently an inhibition of growth rate proportional to the GA_3 concentration. The effect of GA_3 on divisions and growth rate of the haploidal cells of *Chara* thalli, which are able to divide mitotically, depends on the type of cells, on their location in the thallus and on the time action of the regulator. The mitotic activity of the main axis apical cell, expressed by the formation of new nodes, is not, taken as a whole, changed by GA_3, though it may be subjected to some transient modifications. At 10^{-7} M GA_3 concentration, for example, it is initially activated, then slightly inhibited and finally it increases again. At high concentrations (10^{-4} M) GA_3 stimulate the mitotic activity of nodal cells leading to the formation of rhizoids and the divisions of apical cells in resting axial buds, thus accelerating the formation of first nodes on the lateral branches of thalli. The formation of successive nodes on lateral branches is not stimulated by GA_3 similarly as on the main axis of the thallus. It may be assumed then that the action of GA_3 consists in stimulation of the mitotic activity of these cells in which this activity has been temporarily inhibited as a result of apical domination. In *Chara*, this domination is controlled by auxins (Libbert, Jahnke, 1965) and it has the features typical of higher plants (Ducreux, 1974, 1975).

The GA_3 produced diminution of apical dominance in *Chara* thallus is probably connected with an inhibitory influence of this substance on the elongation of internodes. An analogous increase in the number of branches in *Chara* thallus is correlated with a reduction of the internode length — a phenomenon which was also observed under continuous light (Maszewski, 1980). An opposite effect — stimulation of the internode elongation and inhibition of the lateral buds initiation — occur after AMO-1618 application (Table 2), and when the light period is reduced markedly (Maszewski, 1980). In GA_3 treated higher plants the acceleration of the development of axial buds occurs when the stalk growth is inhibited what sometimes occurs after an initial period of stimulation (Marth et al., 1956). In many other cases GA_3 increases rather, than diminishes, the domination of the main shoot and simul-

taneously accelerates its growth rate (ref. Paleg, 1965). The investigations on the effects of GA_3 on different stages of ontogenesis in higher plants show that the obtained effect depends on both age and type of tissue (Simola, Mikkila, 1972; Wielgat et al., 1979). In *Coreopsis*, for example, GA_3 stimulates the elongation rate of young shoots whereas the growth rate of the older ones is inhibited at all GA_3 concentrations used (Simola, Mikkila, 1972). It is connected with the changes in the level of endogenous gibberellins during successive stages of the plant development (El-Antably, 1976; Eckert et al., 1978). The different responses of various cell types of *Chara* thallus to GA_3 application may be due to the changing requirements for gibberellins (what depends on the age and on the physiological stage of a cell), or to a different level of endogenous gibberellins. The presence of the latter in the *Chara* thallus has been shown by Murakami (1966). Inhibition of the growth rate of the generatively matured *Chara* thallus by GA_3 is probably caused by a high level of endogenous gibberellins necessary for the development of generative organs. Such a supposition is suggested by the stimulation effect of the exogenous GA_3 on the development of antheridia and that of oogonia (Godlewski, Kwiatkowska, 1980). The stimulation of internode elongation and the formation of new nodes (Table 2) as well as the retardation of the development of generative organs (Godlewski, Kwiatkowska, unpublished) after the reduction in the level of endogenous gibberellins by AMO-1618 confirms the above supposition.

Acknowledgments

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Wpływ GA_3 i AMO-1618 na rozwój wegetatywnych systemów dojrzałej generatywnie plechy Chara vulgaris L.

Streszczenie

Badano wpływ GA_3 w stężeniach od 10^{-11} do 10^{-4} M oraz AMO-1618 w stężeniach od 10^{-6} do 10^{-4} M na rozwój dojrzałej generatywnie plechy *Chara vulgaris* w ciągu 3-tygodniowej hodowli w warunkach aksenicznych. Stwierdzono, że podziały komórek apikalnych plechy w pąku głównym nie są stymulowane przez GA_3, natomiast w pąkach bocznych są pobudzane w wyższych stężeniach. Dalsza aktywność mitotyczna komórek apikalnych w odgałęzieniach bocznych osi plechy nie jest stymulowana przez GA_3, pobudzane są natomiast pąki boczne tych odgałęzień. W wysokich stężeniach GA_3 przyspieszony jest rozwój chwytników w węzłach młodszych. Wzrost wydłużeniowy wielojądrowych komórek międzywęźli osi głównej i pleurydiów jest hamowany proporcjonalnie do stężeń GA_3. Pod wpływem AMO-1618 zachodzi stymulacja powstawania nowych węzłów, wydłużania międzywęźli oraz opóźnienie pobudzania pąków bocznych i tworzenia się chwytników. Wyniki te sugerują, że hamowanie wydłużania plechy i zmniejszanie dominacji apikalnej przez GA_3 jest spowodowane wysokim poziomem endogennych giberelin w dojrzałej generatywnie plesze.