The influence of gibberellic acid and kinetin on the growth of Scenedesmus quadricauda (Turp.) Bréb.

J. BUCZEK, G. KUBIK-DOBOSZ, and E. TATKOWSKA

Department of Experimental Botany, Institute of Botany, Wrocław University
(Received: January 27, 1975)

## Abstract

The influence of gibberellic acid (GA<sub>3</sub>) and of kinetin (6-furfurylamino purine) on the increment of cell number increase in dry weight and upon protein level in Scenedesmus quadricauda (Turp.) Bréb. was studied. It was found that  $10^{-7}$  M GA<sub>3</sub> stimulates at the same time cell growth and dry weight increase of the algae. No influence of GA<sub>3</sub> upon the protein content was observed. Kinetin of  $10^{-6}$  M concentration stimulates in the initial growth phase cell multiplication and increases the protein level. This substance promotes the increment in dry weight however in the later phase of growth. Furthermore kinetin prolongs the viability of algae, extending the growth phase.

# INTRODUCTION

Investigations concerning the influence and role of higher plant phytohormones upon growth and development of the algae are rather scarce. Hussain and Boney (1969), Jennings (1960), Augier and Hiroshi (1972) and Pedersen (1973) discovered in some species of sea algae cytokinin-like substances. On the other hand, occurrence of gibberelline-like substances was discovered in one species, namely in Ecklonia radiata (Jennings, 1971), Conrad et al. (1959) showed that gibberelline, added to the medium, increased sevenfold the increment of the cell number and freesh weight of Ulotrix subtilissima. Accorinti (1959) found that GA3 stimulated cell division and increased cell size in Scenedesmus obliquus. In spite of the fact that in some instances a positive influence of gibberelline upon the growth of some alga species was observed, gibberelline was not found to be absolutely indispensable for their growth. Pedersen (1968) discovered, on the

other hand, that the culture of the *Thallophyta*, *Ectocarpus fascilatus* and *Pylaiella litoralis*, requires an addition of kinetin to the medium.

The purpose of this work was to determine the influence of gibberelic acid and kinetin on the cell multiplication and dry weight increment in *Scenedesmus quadricauda* cultured in mineral medium.

# MATERIAL AND METHODS

The experiments were conducted with the Scenedesmus quadricauda (Turp.) Bréb. alga, originating from the collection of autotrophic cultures of the Czechoslovakia Academy of Sciences. For culture the medium of Uspenski enriched with 1 ml of microelement solution according to Hoagland per 1 l. medium was used. The medium was prepared from redistilled water containing the following compounds (g/l.):  $Ca(NC_3)_2 \cdot 4H_2O - 0.207$ ,  $KNO_3 - 0.025$ ,  $KH_2PO_4 - 0.025$ ,  $MgSO_4 \cdot 7H_2O - 0.050$ ,  $K_2CO_3 - 0.035$ ,  $Fe_2(SO_4)_3 \cdot H_2O - 0.002$ . The pH of the medium was adjusted to 6.8. The medium was sterilized for 45 min at overpressure of 0.75 Atm.

The effect of the substances in question on alga growth was tested in 100-ml Erlenmeyer flasks. Into every flask 25 ml of the medium containing the investigated substances was poured. The cultures were inoculated under sterile conditions with 1 ml of alga suspension taken from the stock culture. This culture was obtained by transferring every 15 days of 4 ml of alga suspension to a 300-ml flask with 100 ml of the medium. Inoculation was done in the 15<sup>th</sup> day of basic culture. Test experiments were carried out in 5 replications for each combination and for each concentration of the studied substances. Each experiment was repeated at least three times. The results are given as means of the 5 replications. The experiments were performed in a photostatic room where the light was provided by fluorescent lamps. Light intensity during culture was 5000 lux and temperature 24—26° C.

Dry weight was determined by filtering the alga suspension through a Synpor membrane filter, washing it on the filter with 0.01 N HCl and with distilled water. The mass was dried at  $65\,^{\circ}\text{C}$  and then determined by weight.

The quantity of cells (four membered unicellular colonies) was determined by counting in a Fuchs-Rosenthal chamber. Bicellular colonies and single cells were considered, respectively as  $^{1}/_{2}$  and  $^{1}/_{4}$  of a colony. The numbers of cells are given as arithmetic means from 4 replications with reference to  $^{1}/_{32}$  ml. Then, from the so obtained means, from 5 replications of the same combination, once more an arithmetic mean was calculated. This mean is the final numerical data characterizing the quantity of cells in the given combination.

The content of soluble protein in the algae was determined as follows: 25 ml of alga suspension was filtered, washed quantitatively into 10-ml test tubes and centrifuged. The sediment was treated with 2 ml of hot 6 per cent trichloroacetic acid and then extracted hot for 1 minute. After centrifuging, the supernatant was discarded. To the sediment 2 ml of 1 N NaOH was added and tubes was placed on a boiling water bath for 10 minutes. After cooling the solution was made up to 5 ml with 1 N NaOH and centrifuged. Protein in the supernatant was determined by the method of Lowry et al. (1951).

Statistical calculations were carried out by the Snedecor "F" test for a confidence level P = 0.05 (Ulińska, 1957; Oktaba, 1966).

# RESULTS

The results of the experiments (results shown in Table 1), were aimed at finding the eventual influence of gibberelline and kinetin upon the growth of S. quadricauda algae, and at determination of the effect of concentration of those substances. The results indicate that GA<sub>3</sub> as well kinetin lead to an increment in dry weight of algae after 12 days of

Table 1

Effect of GA<sub>3</sub> and kinetin concentration on dry weight content in 12 day growth of S. quadricauda cultures

		mg/25 ml of medium
Control	1,22	12.5
$GA_3 = 10^{-6} M$		12.5
$5 \times 10^{-7} \text{ M}$		18.5*
10 <sup>-7</sup> M		17.3*
10 <sup>-8</sup> M	~	13.8
Control		11.1
Kinetin 10 <sup>-4</sup> M	-	13.0
10 <sup>-5</sup> M		12.5
10 <sup>-6</sup> M		14.5*
$10^{-7} M$		13.6
10 <sup>-8</sup> M		12.7

<sup>\*</sup> Differences significant

culture. Under the conditions of our cultures this moment is closest to the end of the intensive alga growth phase. It was found, that from among the GA<sub>3</sub> concentrations tested  $5 \times 10^{-7}$  M and  $10^{-7}$  M and as regards kinetin  $10^{-6}$  M and  $10^{-7}$  M evoked stimulation. In the following experiments the authors used the concentrations determined in the experiment described above.

In Table 2 are listed the results of alga yield measured by the increment of dry weight after 6, 12, 18 days of culture and the content of soluble protein. The results indicate that  $10^{-7}$  M GA<sub>3</sub> significantly stimulates the increment of alga dry weight after 6 and 12 days of culture, but gives no significant increment of dry weight after end of the culture (after 18 days). On the other hand, GA<sub>3</sub> did not influence protein content on the particular days of measurement.

Table 2

Effect of gibberellin and kinetin on the increase in dry weight and content of protein. The results represent the average value of dry weight and protein for five samples per series

		Incubation time in days:						
	6	6		12		18		
	mg/25 ml of medium							
	dry weight	protein	dry weight	protein	dry weight	protein		
Control	6.80	2.31	17.40	2.64	21.40	2.86		
$GA_3 5 \times 10^{-7} M$	8.00	2.40	18.80	2.86	23.20	2.86		
$GA_3   10^{-7} M$	8.70+	2.57	22.20*	2.93	25.20	2.74		
Control	4.90	2.02	14.20	2.15	19.90	2.47		
Kinetin 10 <sup>-6</sup> M	5.60	2.59*	17.50*	2.60*	24.20*	2.77		
Kinetin 10 <sup>-7</sup> M	4.90	2.49*	17.30*	2.49	22.30	2.77		

<sup>\*</sup> Differences significant

The reaction of the algae to kinetin was different. In the initial growth phase (after 6 days) the dry weight yield in the presence of kinetin did not differ from the control values. After 12 days of culture the increment of dry weight of the algae under the influence of 10<sup>-6</sup> M and 10<sup>-7</sup> M kinetin was on the average 22 per cent greater than in the control. In the final effect, however, only 10<sup>-6</sup> M kinetin significantly increased the increment of the algae dry weight by about 21 per cent as compared with the control. Addition of kinetin to the medium also increased the protein content, especially in the initial phase of algae growth. At the end of the culture, however, the protein content in the combination with kinetin was the same as in the control.

The algae cells counts on particular days are given in Table 3. It is seen from the data that an addition of  $10^{-7}$  M GA<sub>3</sub> to the medium increased the alga yield when measured by the number of cells after 6 and 12 days of culture. In the final effect, however (after 18 days) the number of the algae cells in the presence of GA<sub>3</sub> did not increase significantly as compared with the control.

Kinetin of  $10^{-6}$  M concentration distinctly enhanced cell multiplication on all days by more than 30 per cent of the average. At this kinetin concentration a significant acceleration of the rate of cell division was

Table 3

Effect of gibberellin and kinetin on the number of Scenedesmus quadricauda cells. The results represent the average values for five samples per series

	Incubation time in days					
	6	12	18			
	The number	The number of cells in 1/32 ml of medium				
Control	3090	3692	4900			
$GA_3 5 \times 10^{-7} M$	3128	3921	5058			
$GA_3 = 10^{-7} M$	3850*	4401*	5304			
Control	3274	4000	4644			
Kinetin 10 <sup>-6</sup> M	4250*	5527*	6116*			
Kinetin 10 <sup>-7</sup> M	2982	4574	6552*			

<sup>\*</sup> Differences significant

noted throughout the whole duration of the culture. Lower kinetin concentrations ( $10^{-7}$  M) enhanced cell division not earlier than after 12 days of culture and caused a prolongation of intensive algae growth.

#### DISCUSSION

The results presented in the previous chapter indicate that  $GA_3$  and also kinetin in certain definite concentrations stimulate dry weight increment as well cell division of S. quadricauda. However the presence of both investigated substances was not found to be absolutely necessary for the growth of S. quadricauda, although in the literature there are data indicating the indispensability of kinetin for the culture of sea algae from the Thallophyta group (Pedersen, 1968). The present results show also differences in the way the two studied substances act on the cultures.

Gibberelline, added to the medium, stimulated at he same time the cell multiplication and increased the increment of dry weight only in the intensive growth phase. After the end of this phase the yield of mass as well as the quantity of the cells in the presence of  $GA_3$  were identical as those in the control.

The results here obtained show that  $GA_3$  stimulates cell division as well as increase in dry weight. Similar results were achieved by Accorinti (1959), who discovered a stimulating influence of  $GA_3$  upon cell division in Scenedesmus quadricauda and by Conrad et al. (1959) who found a sevenfold increase in wet weight increment and cell division in Ulotrix subtilissima, under the influence of  $GA_3$  added to the medium. Provasoli (1957) on the other hand, observed an increase of the cell number due to  $GA_3$  in Ulva lactuca.

The investigations of the cited and the present authors indicate the stimulating influence of GA<sub>3</sub> upon growth processes in some species of algae.

If we compare the influence of kinetin upon the alga growth with that of GA<sub>3</sub> a different character of the action of these two substances is seen. While GA<sub>3</sub> stimulated cell division and simultaneous dry weight increment, kinetin affected in the initial growth stage, only cell division. The influence of kinetin upon the dry weight increment become noticeable not earlier than after 12 days of culture. The fact that the influence of GA<sub>3</sub> upon alga mass increment and the quantity of the cells is not significant towards the end of the experiment (after 18 days), but that kinetin prolongs the ability of cells to divide, extending the intensive growth phase, points to a different mechanism of action of the studied substances. The above presumptions are also confirmed by the measurements of protein content. Gibberelline as compared with the control did not cause any significant changes in the protein level, but kinetin exerted a significant influence upon the increase of protein and at the same moment when an increment of the cell number was observed.

Therefore, it may be presumed, that the first effect of kinetin is stimulation of cell multiplication and of an increase in soluble protein content, while the observed increment in dry weight is a secondary proces. Kinetin also prolongs the viability of the *Scenedesmus quadricauda* cells by increasing their ability to division. On the other hand, gibberelline stimulates cell division and the increase in dry weight at the same time. Its influence is significant only in the intensive growth phase of algae.

### REFERENCES

Accordnti J., 1959 [In:] Phinney B.O. and West Ch. A., Enc. of Plant Physiol. 1961. Springer. Berlin.

Augier H., & Hiroshi H., 1972. C. R. Acad. Sci. 16: 1765-68.

Conrad H., Saltman P., and Eppley R., 1959. Nature, 184: 556-57.

Hussain A., & Boney A. D., 1969. Nature, 223: 504-5.

Jennings R. C., 1969. Austr. J. Biol. Sci. 22: 621—27.

Jennings R. C., 1971. Austr. J. Biol. Sci. 24: 1115-24.

Lowry O. H., Rosenbrough N. J., Farr A. L., and Randall R. J., 1951.
J. Biol, Chem. 193: 165—75.

Oktaba W., 1966. Elementy statystyki matematycznej. PWN. Łódź.

Pedersen M., 1968. Nature. 218: 776.

Pedersen M., 1973. Plant Physiol. 28: 101-5.

Provasoli L., 1957. Biol. Bull. 114: 375-84.

Ulińska M., 1957. Technika obliczeń. PWRiL. Warszawa.

Author's address:
Doc. dr hab. Józef Buczek
mgr Genowefa Kubik-Dobosz
dr Ewa Tatkowska
Institute of Botany, Wrocław University;
Kanonia Str. 6/8; 50-328 Wrocław; Poland

Wpływ kwasu giberelinowego i kinetyny na wzrost glonów Scenedesmus quadricauda \*

## Streszczenie

Badano wpływ kwasu giberelinowego (GA<sub>3</sub>) oraz kinetyny (6-furfuryloamino puryny) na przyrost ilości komórek, przyrost suchej masy i poziom białka ekstrahowanego u *Scenedesmus quaricauda* (Turp.) Bréb. Stwierdzono, że spośród badanych stężeń 10<sup>-7</sup> M GA<sub>3</sub> stymuluje równocześnie przyrost ilości komórek i suchej masy glonów. Stymulacja ta zaznacza się jednakże jedynie w fazie intensywnego wzrostu glonów. Nie zaobserwowano wpływu GA<sub>3</sub> na zmianę poziomu białka. Kinetyna w stężeniu 10<sup>-6</sup> M stymuluje w początkowej fazie wzrostu namnażanie się komórek oraz zwiększa poziom białka w glonach. Wpływ kinetyny na przyrost suchej masy zaznacza się natomiast w późniejszej fazie wzrostu glonów. Kinetyna ponadto przedłuża żywotność glonów wydłużając fazę wzrostową.

Uzyskane wyniki wskazują na różnice w sposobie działania gibereliny i kinetyny na wzrost S. quadricauda.

<sup>\*</sup> Praca finansowana przez Politechnikę Wrocławską w ramach Programu Badawczego "Ochrona Środowiska".