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Effect of phytohormones on the growth of *Scenedesmus quadricauda* (Turp.) Bréb.

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Abstract

Studies were made on the effect of indole-3-acetic acid (IAA), gibberellic acid (GA), kinetin (Kin), and various combinations of these substances upon dry matter increments, soluble protein content, and chlorophyll levels in the cultures of *Scenedesmus quadricauda* (Turp.) Bréb. It was found that all these phytohormones, added separately to the medium, stimulated dry matter increment and protein content, although their effect depended on the concentration and the duration of culture. Addition of various combinations of the substances under study into the medium resulted in a less pronounced increment of dry matter, whereas the content of soluble protein significantly increased. The results reflect positive reaction of *Scenedesmus quadricauda* to the addition of phytohormones into the medium. They also suggest some differences in the action of particular hormones.

INTRODUCTION

In an earlier paper (Buczek et al., 1975) the effect of gibberellic acid (GA) and kinetin on *Scenedesmus quadricauda* (Turp.) Bréb. growth was reported. We found that GA stimulated the increment of dry matter and cell number. Simultaneously, kinetin induced cell multiplication in the initial growth phase, however the increase of dry matter was affected in the later growth phase o cultures. The effect of applied plant hormones on the growth and development of sea and marine algae has been reported by several authors (Conrad et al., 1959; Ahmad, Winter, 1968 a, b; Ahmad, 1971, 1973; Buczek et al., 1975; Hřib, 1975; Augier, 1976 a, b), although the effect of applied indole-3-acetic acid (IAA) and various combinations of IAA, GA, and kinetin on the growth of *S. quadricauda* has not been studied as yet. In the present experiments the effect of plant hormones, applied separately or in com-

binations, on the increment of dry matter, soluble protein content, and the level of chlorophyll was determined to provide further information on the effect of phytohormones on the growth of *S. quadricauda*.

MATERIAL AND METHODS

The experiments were conducted on *Scenedesmus quadricauda* (Turp.) Bréb. strain 119, purchased from the collection of autotrophic cultures of the Czechoslovakian Academy of Sciences. The culture methods and growth medium (Uspenski) were the same as described previously (Buczek et al., 1975), with some modifications. The cultures were grown in a climatic chamber under illumination of 7000 lx provided by fluorescent tubes. The temperature was kept at 22 ± 1°C. The cultures were grown under 17 : 7 h light and darkness respectively, and shaked manually 3 or 4 times a day. Gibberellic acid (GA), indole-3-acetic acid (IAA), and kinetin (Kin) were obtained from "Sigma". The tested plant hormones were added immediately to the basal medium.

Dry matter was determined by filtration of culture suspension through a "Synpor 5" membrane filter, washing it on the filter with 0.01 N HCl and distilled water, and dried at 65° C. The soluble protein in algae was extracted as described previously (Buczek et al., 1975), and the protein was determined by Lowry et al. (1951) method.

Chlorophyll was extracted with acetone from 5-10 ml of cell suspension, after grinding the washed and centrifuged culture suspension with 90% acetone. The extraction was repeated 2-3 times. The acetone soluble pigments were separated from colorless debris by centrifugation for 10 min at 1800 × g. Absorbance was determined at 664 and 647 nm using a VSU-2P (Carl Zeiss-Jena) spectrophotometer. Chlorophyll was estimated according to Jeffrey and Homphrey (1975) using equation for higher plants and algae.

The results are given as means of 5 replications. Each experiment was repeated at least three times. Statistical calculations were made by the method of variance analysis for unidirectional classification according to Ulińska (1957).

RESULTS

The effect of phytohormones upon the growth of *S. quadricauda* is presented in Table 1. These results suggest that all substances under study stimulated the increment of dry matter, although their effectiveness

depended on the concentration. Gibberellin significantly increased the increment of dry matter of the algae if used at concentrations of 5×10^{-7} M and 10^{-7} M. The effect of GA was visible after 7 and 14 days of culture growth. Auxine significantly stimulated the increment of dry matter in all concentrations applied, but its effect was visible only after 14 days of growth. Kinetin in concentrations of 10^{-7} M and 5×10^{-7} M visibly increased dry matter in later growth phase, but at the end of the experiment its effect was rather weak.

Table 1

Effect of gibberellic acid (GA), indole-3-acetic acid (IAA) and kinetin (Kin) on the growth of *Scenedesmus quadricauda*

Concentration of phytohormones	Growth period in days		
	7	14	21
	mg dry weigh per 25 ml of medium		
Control	4.36 a	15.59 c	33.02 e
GA 10^{-6} M	4.43 a	16.19 c	31.78 e
GA 5×10^{-7} M	5.61 b (129)	20.29 d (130)	37.78 e
GA 10^{-7} M	5.49 b (126)	19.73 d (127)	36.90 e
IAA 10^{-6} M	4.48 a	20.03 d (128)	37.50 e
IAA 5×10^{-7} M	4.62 a	19.60 d (126)	37.98 e
IAA 10^{-7} M	5.11 a	20.78 d (123)	30.22 e
Kin 10^{-6} M	3.91 a	15.35 c	31.78 e
Kin 5×10^{-7} M	4.73 a	18.90 d (121)	30.22 e
Kin 10^{-7} M	5.15 a	19.50 d (125)	37.58 e

Values in brackets reflect the effect of treatment in relation to the control. Differences between values denoted with different letters are significant at the 0.05 probability level.

Table 2 presents the results with respect to soluble protein content after 7, 14, and 21 days of growth of *S. quadricauda* in cultures. All concentrations of GA increased the increment of soluble protein after 7 days of algal growth. After 14 days significant increase of the soluble protein content was noted only for GA concentration of 10^{-7} M. Addition of kinetin at concentrations of 10^{-7} M and 5×10^{-7} M also increased the content of soluble protein after 7 and 14 days of culturing. Stimulating effect of auxine upon soluble protein content was noted only after 14 days of algae growth, in medium containing 10^{-6} M IAA. At the end of culture growth none of the hormones under study stimulated the increment of protein as compared to the control.

Chlorophyll content per unit of dry weight is presented in Table 3. Total chlorophyll content in algae cells growing on the medium with an addition of GA was significantly higher compared to the control on all days of the measurements. It was found that in the presence of

Table 2

Effect of gibberellic acid (GA), indole-3-acetic acid (IAA) and kinetin (Kin) on the content of soluble protein in *S. quadricauda* cultures

Concentration of phytohormones		Growth period in days		
		7	14	21
		mg protein per 25 ml. of medium		
Control		0.49 a	2.25 e	3.43 f
GA	10^{-6} M	0.59 b (120)	2.50 e	3.55 f
GA	5×10^{-7} M	0.79 c (161)	2.90 f (129)	3.17 f
GA	10^{-7} M	0.61 b (124)	2.87 f (127)	3.07 f
IAA	10^{-6} M	0.55 a	3.30 f (128)	2.90 f
IAA	5×10^{-7} M	0.45 a	2.77 f (123)	3.17 f
IAA	10^{-7} M	0.52 a	2.77 f (123)	2.90 f
Kin	10^{-6} M	0.49 a	2.60 e	3.50 f
Kin	5×10^{-7} M	0.90 d (183)	3.07 f (136)	3.30 f
Kin	10^{-7} M	0.77 c (157)	2.97 f (132)	3.80 h

Values in brackets reflect the effect of the treatment in relation to the control. Differences between values denoted with different letters are significant at the 0.05 probability level.

10^{-6} M GA there was a simultanous increase of the content of chlorophyll *a* and *b*, while at a concentration of 10^{-7} M gibberellin stimulated the content of chlorophyll *b* mostly. In the presence of IAA there were no changes of chlorophyll pigments, nor of the ratio of chlorophyll *a* to chlorophyll *b*. Kinetin affected changes of the total chlorophyll content in a rather insignificant way, but the ratio between chlorophyll *a* and *b* was relatively high in the cells treated with kinetin after 7 and 14 days of growth.

A series of experiments were undertaken in order to study the effect of various combinations of GA, IAA, and Kin upon growth and protein content in the cells of *S. quadricauda*. The results are given in Tables 4 and 5. As it is seen from these data, dry matter increments and protein content were stimulated by various combinations of the hormones. And thus, an addition of GA and kinetin to the medium stimulated the increment of dry matter and protein content only after 14 days of the growth of algae. Nevertheless, concentration of 10^{-7} M GA in the combination with 10^{-6} M Kin significantly increased the soluble protein content on all days of the measurements. Auxine added to the medium together with kinetin, in various quantitative combinations, significantly increased the increment of dry matter and soluble protein content after 7 and 14 days of culturing, with the exception of the combination 10^{-7} M IAA + 10^{-7} M Kin. The effect of the combination IAA + GA on the increment of dry matter was insignificant, although the addition of 10^{-6} M IAA + 10^{-7} M GA significantly increased the content of protein on all days of the measurements.

Table 3

Influence of GA, IAA and kinetin (Kin) on the amount of chlorophyll (Chl) content (in mg/g dry wt.) in *Scenedesmus quadricauda*

Treatment	Growth period in days											
	7				14				21			
	Chl a	Chl b	Total Chl	Chl a / Chl b	Chl a	Chl b	Total Chl	Chl a / Chl b	Chl a	Chl b	Total Chl	Chl a / Chl b
Control	6.6	2.3	8.9	2.8	3.6	1.8	5.4	2.0	1.3	0.2	1.5	5.9
GA 10^{-6} M	9.9	3.1	13.0	3.2	4.1	2.5	6.6	1.6	1.4	0.6	2.0	2.3
5×10^{-7} M	6.9	2.9	9.8	2.3	3.1	2.0	5.1	1.5	1.4	0.5	1.9	2.8
10^{-7} M	6.9	3.1	10.0	2.2	3.5	3.1	6.6	1.1	1.4	0.4	1.8	3.5
IAA 10^{-6} M	8.2	2.2	10.4	3.6	4.1	2.1	6.2	1.9	1.3	0.3	1.6	4.6
5×10^{-7} M	8.6	2.6	11.2	3.3	4.1	1.5	5.6	2.7	1.4	0.3	1.7	4.7
10^{-7} M	6.5	2.2	8.7	2.9	3.8	1.7	5.5	2.2	1.4	0.3	1.7	4.7
Kin 10^{-6} M	7.3	1.6	8.9	4.6	3.9	1.4	5.3	2.8	2.0	0.5	2.5	4.3
5×10^{-7} M	7.3	1.5	8.8	4.9	4.2	1.4	5.6	3.0	1.4	0.8	2.2	1.8
10^{-7} M	6.4	1.5	7.9	4.3	4 5	1.3	5.8	3.5	1.2	0.5	1.7	2.6

Table 4

Effect of various combinations of phytohormones on the growth of *Scenedesmus quadricauda*

Concentration of phytohormones			Growth period in days		
			7	14	21
GA		Kinetin	mg dry weight per 25 ml of medium		
—		—	9.07 a	19.11 c	33.80 e
10^{-6} M	+	10^{-6} M	10.43 a	27.14 d (142)	36.17 e
5×10^{-7} M	+	5×10^{-7} M	9.16 a	23.50 d (123)	33.09 e
10^{-7} M	+	10^{-7} M	9.70 a	20.45 c	34.14 e
10^{-7} M	+	10^{-6} M	8.79 a	24.46 d (128)	32.45 e
IAA		Kinetin			
10^{-6} M	+	10^{-6} M	11.52 b (127)	24.84 d (138)	34.48 e
5×10^{-7} M	+	5×10^{-7} M	12.69 b (140)	24.46 d (128)	34.48 e
10^{-7} M	+	10^{-7} M	11.52 b (127)	23.69 d (124)	35.49 e
10^{-7} M	+	10^{-6} M	8.52 a	24.08 d (126)	33.12 e
IAA		GA			
10^{-6} M	+	10^{-6} M	9.25 a	20.26 c	39.20 e
5×10^{-7} M	+	5×10^{-7} M	9.61 a	20.26 c	35.82 e
10^{-7} M	+	10^{-7} M	8.79 a	21.78 c	33.80 e
10^{-6} M	+	10^{-7} M	9.79 a	23.50 d	33.12 e

Values in brackets reflect the effect of the treatment in relation to the control. Differences between values denoted with different letters are significant at the 0.05 probability level.

Table 5

Effect of various combinations of phytohormones on the content of soluble protein in *S. quadricauda* cultures

Concentration of phytohormones			Growth period in days		
			7	14	21
GA		Kinetin	mg protein per 25 ml medium		
—		—	1.02 a	2.76 b	3.61 d
10^{-6} M	+	10^{-6} M	1.22 a	3.80 d (138)	3.89 d
5×10^{-7} M	+	5×10^{-7} M	0.98 a	3.56 d (129)	3.57 d
10^{-7} M	+	10^{-7} M	0.94 a	3.39 d (123)	3.79 d
10^{-7} M	+	10^{-6} M	2.49 b (244)	3.67 d (133)	6.73 e (186)
IAA		Kinetin			
10^{-6} M	+	10^{-6} M	11.52 b (127)	24.84 d (130)	34.48 e
5×10^{-7} M	+	5×10^{-7} M	12.69 b (140)	24.48 d (128)	34.48 e
10^{-7} M	+	10^{-7} M	11.52 b (127)	23.69 d (124)	35.49 e
10^{-7} M	+	10^{-6} M	8.52 a	24.08 d (126)	33.12 e
IAA		GA			
10^{-6} M	+	10^{-6} M	9.25 a	20.26 c	39.20 c
5×10^{-7} M	+	5×10^{-7} M	9.61 a	20.26 c	35.82 e
10^{-7} M	+	10^{-7} M	8.79 a	21.78 c	33.80 e
10^{-6} M	+	10^{-7} M	9.79 a	23.50 d	33.12 e

Values in brackets reflect the effect of the treatment in relation to the control. Differences between values denoted with different letters are significant at the 0.05 probability level.

DISCUSSION

The results show that both gibberellin and auxine, as well as kinetin, stimulate the increment of dry matter, the content of soluble protein, and the content of assimilation pigments in the cells of *S. quadricauda*. Activity of particular growth substances depended on their concentration in the medium, the range of active concentrations being between 10^{-6} and 10^{-7} M. However, there were several significant differences in the way of acting of particular substances although the reaction of the algae was more or less similar.

Gibberellin in the medium stimulated both the increment of dry matter and the content of soluble protein in the first phase of the growth of algae. After 3 weeks of culturing the effect of gibberellin was visible only with respect to the total chlorophyll content whereas the content of protein and dry matter of the algae did not differ from the control. It was stated in the previous paper (Buczek et al., 1975) that in the initial phase of *S. quadricauda* growth GA stimulates also the division of cells. Hence, it may be assumed that this hormone plays some function in the processes of the development of algae. Similar suggestion was given by Augier (1976 b) who stated that gibberellin stimulated developmental processes in some species of algae. Also other authors noted positive reaction of algae to the addition of gibberellin into the medium. Accorinati (1959) noted stimulating effect of GA upon the divisions of *S. quadricauda* cells, Saona (1964) — the effect of GA upon the increments of dry matter in *S. qaudricauda, S. obliquus, Chlorella vulgaris,* and *Ch. pyrenoidosa*, while Conrad et al. (1959) — upon both — cell divisions and increments of dry matter in *Ulotrix subtilissima*. On the other hand, Ahmad (1973) did not find any stimulating effect of GA on the increments of matter in *Anacystis nidulans* and *Chlorogloea fritschii*.

The effect of kinetin visibly differed from that of GA. The latter substance stimulated the increments of dry matter only in the later phase of growth (after 14 days), whereas the content of soluble protein increased in the initial phase of culture and remained relatively high on the successive days of the measurements. Total content of chlorophyll pigments did not differ from the control, but the ratio between chlorophyll *a* and *b* visibly increased. These data may suggest different mechanism of the action of kinetin and GA. This statement is also supported by the earlier work (Buczek et al., 1975) in which it was found that kinetin prolonges the vitality of *S. quadricauda* by the prolongation of the growth phase. Furthermore, it visibly affects cell divisions both in the initial and later phases of culturing. On the other hand gibberellin stimulated cell divisions in the initial phase of culturing

(Buczek et al., 1975). The effect of kinetin on cell divisions in *Spirogyra longata* was also noted by Olszewska (1958), whereas Ahmad (1973) noted increments of cell mass in *Anacystis nidulans* under the effect of kinetin.

The effect of auxine upon the increments of cell mass of *S. quadricauda* was similar as in case of kinetin, being noticeable only after 14 days of culture growth. At the same time it correlated with the increase of soluble protein. However, the fact that in the initial phase of the growth of algae in the presence of IAA the protein content did not differ from the control suggests that kinetin and auxine differ as regards the mechanisms of their action. Furthermore, it was found that IAA did not affect the chlorophyll content, nor the ratio between both forms of chlorophyll. Somewhat different results on the effect of IAA upon the growth of some species of green and blue-green algae were obtained by Ahmad and Winter (1968 a, b), and Ahmad (1971). These authors noted stimulating effect of IAA on the growth (measured by dry matter increments) both in short and long-term experiments. Dependence between growth stimulation and IAA concentration in the medium (in the range of 10^{-9} to 10^{-7} M) was also noted. Hence, it seems that auxine actively affects growth of several species of algae, and its effectiveness depends on the concentration and phase of algal growth.

Various combinations of optimal concentrations of GA and kinetin had a somewhat less pronounced effect upon the increments of the mass of S. *quadricauda*. Nevertheless, combination of 10^{-7} M GA + 10^{-6} M kinetin effectively stimulated the increments of soluble protein on all days of the measurements. It may be assumed that the growth of *S. qaudricauda* cultures in the presence of gibberellin and kinetin (at proper concentration) almost doubles the content of soluble protein. Similar effect was noted in the presence of 10^{-6} M kinetin + 10^{-7} M IAA, but the total content of soluble protein was lower than in case of GA + Kin. Auxine combined with kinetin significantly stimulated the increments of the cell dry matter. Ahmad (1973) noted significant effect of IAA + GA on the growth of mass in *Chlorogloea fritschii*, but this fact was not confirmed in successive experiments with *S. quadricauda*, although both substances, in concentrations of 10^{-7} M and 10^{-7} M GA + 10^{-6} M IAA significantly affected the increments of soluble protein content in this species. These results suggest positive reaction of *S. quadricauda* to the addition of phytohormones or their combinations into the medium. They also point to differentiated action of particular hormones.

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Wpływ fitohormonów na wzrost Scenedesmus quadricauda (Turp.) Bréb.

Streszczenie

Badano wpływ kwasu indolilo-3-octowego (IAA), kwasu giberelinowego (GA) i kinetyny (Kin.) oraz różnych kombinacji tych substancji na przyrost suchej masy, zawartość białka rozpuszczalnego i chlorofilu w kulturach *Scene-*

desmus quadricauda (Turp.) Bréb. Stwierdzono, że wszystkie testowane fitohormony podane pojedynczo do pożywki stymulowały przyrost masy i zawartość białka, jednakże ich efekt był zależny od stężenia i wieku kultury. Dodanie do pożywek różnych kombinacji badanych substancji w małym stopniu wpływało na przyrost masy, natomiast istotnie zwiększało zawartość białka rozpuszczalnego. Uzyskane wyniki wskazują na pozytywną reakcję *Scenedesmus quadricauda* na dodatek do pożywki hormonów roślinnych oraz sugerują pewne różnice w sposobie ich działania.