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Phytohormone Effects on Cell Division in *Chlorella pyrenoidosa* Chick (TX-7-11-05) (Chlorellaceae)

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Abstract. Phytohormone effects on cell division of synchronous cultures of *Chlorella pyrenoidosa* (TX-7-11-05) were studied under different photo flux densities. The time required for initiation of incipient cell division was reduced significantly by treatment with kinetin (6-furfurylaminopurine), gibberellic acid, and indole-3-acetic acid. Data show that kinetin was more effective than gibberellic acid, which was more effective than indole-3-acetic acid. Studies with combinations of the phytohormones revealed no antagonistic, additive, or synergistic effects.

Results of studies related to effects of phytohormones in algae prior to 1962 have been summarized by Conrad and Saltman (1962). Provasoli and Carlucci (1974) reviewed much of the work since 1962, and Taylor and Wilkinson (1977) addressed the occurrence and possible roles of gibberellins in algae.

Endogenous levels of indole-3-acetic acid (IAA) have been assessed in *Chlorella pyrenoidosa* (Mowat 1965, Grotbeck and Vance 1972) and in *Oscillatoria* sp. and *Orchromonas* sp. by Mowat (1965). Arendarchuk (1978) reported the isolation of IAA from *Microcystis, Aphanizomenon,* and *Phormidium*. The existence of endogenous IAA in several algae has been questioned by Buggeln and Craigie (1971) and Buggeln (1976).

A positive growth response of algae to the exogenous application of IAA was reported by Adhikary and Pattnaik (1978). Wood and Berliner (1979) reported that IAA treatment increased the rate of cell division in *Micrasterias thomasiana* by 50% at pH 6.8. Autocolony formation in *Scenedesmus quadricauda* was increased by IAA treatment (Monoson et al. 1979).

Bralczyk et al. (1978) found that the growth of *Euglena gracilis* was accelerated by GA during experimental growth. Growth stimulation in blue-green and green algae also was reported by Czerpak (1979), and Tatkowska and Buczek (1980) found that treatment of *Scenedesmus quadricauda* with GA, IAA, or a

cytokinin resulted in an increase in dry weight and total protein. The marine diatom *Cyclotella cryptica* responded to GA treatment (2×10^{-5} $\mu g \cdot ml^{-1}$) with a shorter lag phase of growth and an increased total number of cells produced (Adair and Miller 1982).

Cytokinins have also been reported to ellicit positive responses in algae (Kim and Greulach 1961, Jennings 1969, Ahmad 1973). Three ribonucleosides isolated from *Euglena gracilis* var. *bacillaris* tRNA by Swaminathan and Bock (1977) were reported to be the site of cytokinin activity. Mating activity was increased by *Chlamydomonas* sp. (Ishiura 1976). High concentrations of 6-furfurylaminopurine (0.01–1.0 $mg \cdot ml^{-1}$) eliminated the lag phase of *Cosmarium botrytis* cultures at pH 5.3 and 8.0 (Berliner 1981).

Growth of *Scenedesmus quadricauda* was significantly increased by ethylene (applied as Ethephon or 2-chloroethylphosphonic acid) at concentrations of 0.001–1.0 $mg \cdot ml^{-1}$ and RNA levels were significantly higher in cells treated with 0.1 $mg \cdot ml^{-1}$ Ethephon (Chapman and Vance 1976). Recently, Huang and Chow (1984) isolated a strain of the blue-green alga, *Hapalosiphon*, that produces ethylene.

The present study was done to determine the effects of IAA, GA, and K on cell division of *Chlorella pyrenoidosa* TX-7-11-05 maintained in synchronous culture.

Materials and Methods

Synchronous cultures of *Chlorella pyrenoidosa* Chick. TX-7-11-05 (UTEX 1230) were obtained and grown axenically as detailed in Grotbeck and Vance (1972). Cultures were grown under a continuous flux density of 50, 100, or 180 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Filter-sterilized phytohormones IAA (indole-3-acetic acid), GA (gibberellic acid), and K (6-furfurylaminopurine) were added to media (final volume 25 ml) contained in 125-ml Erlenmeyer flasks. Each phytohormone was prepared in a final concentration of 10.0, 1.0, and 0.1 $\mu g \cdot ml^{-1}$. Each flask was inoculated with 10^7 cells from the synchronized stock cultures at the end of a dark period, when ~99% of the cells were small daughter cells. Each treatment, including a control, was run in triplicate. Treatment cultures were grown under the conditions stated above. Beginning with the time of transfer, observations were made at 30-min intervals until the time of incipient cell division (Sorokin and Krauss 1965). Because of the large number of observations required at each 30-min interval, 1 ml of cell suspension was aseptically withdrawn from each flask and preserved in 2% Formalin. The cell counts and other observations were then completed as time permitted. The entire experiment was repeated three times.

To determine if additive or synergistic effects occurred, all possible combinations of the three phytohormones, each at 1.0 and 10.0 $\mu g \cdot ml^{-1}$, were tested in the manner described.

Table 1. Statistical analysis of phytohormone effects on incipient cell division in synchronized cultures of *Chlorella pyrenoidosa* TX-7-11-05.

	Photon flux density ($\mu E \cdot m^{-2} \cdot s^{-1}$)					
	50		100		180	
Phytohormone	$\bar{X}$ time (min)	Conc. ($\mu g \cdot ml^{-1}$)	$\bar{X}$ time (min)	Conc. ($\mu g \cdot ml^{-1}$)	$\bar{X}$ time (min)	Conc. ($\mu g \cdot ml^{-1}$)
IAA	369	10	376	0	456	0.1
	362	1	365	1	421	1
	362	0	363	10	411	0
	358	0.1	362	0.1	408	10
	(p = 0.0036)		(p = 0.0001)		(p = 0.1985)	
GA	369	0.1	376	0	431	0.1
	362	0	347	0.1	417	1
	357	1	346	10	411	0
	347	10	343	1	387	10
	(p = 0.0001)		(p = 0.0001)		(p = 0.0001)	
K	369	0.1	376	0	411	0
	362	0	348	0.1	406	0.1
	347	1	332	1	395	1
	316	10	299	10	341	10
	(p = 0.0001)		(p = 0.0001)		(p = 0.0001)	

Time is the average time of 3 experiments to incipient cell division for each concentration of each phytohormone. Treatments grouped together with a bar are not significantly different (Duncan's multiple-range test, α = 0.05). p Values are probabilities from parametric ANOVA.

Results and Discussion

The degree of synchrony exceeded 99%. Cell division began immediately after the light cycle and was completed by 3 h into the dark phase.

Statistical analyses of the data showed that the high-temperature strain of *Chlorella* is significantly affected by K and to a somewhat lesser extent by GA and IAA (Table 1). The time to incipient cell division was reduced by K under all photon flux densities and all concentrations employed. The cytokinins have long been known as cell division promoters in higher plants (Leonard et al. 1968, Horgan 1984), and my data suggest they are operative in the algae also. This conclusion is supported by Berliner's (1981) finding that the synchronous generation time of *Cosmarium botrytis* was reduced owing to the elimination of an initial lag phase by treatment with high concentrations of K (10–1000 $\mu g \cdot ml^{-1}$).

Under photon flux densities of 50 and 180 $\mu E \cdot m^{-2} \cdot s^{-1}$, a concentration of 10 $\mu g \cdot ml^{-1}$ GA significantly reduced the time to cell division of *Chlorella* relative to the controls and the lower concentrations of GA. Under 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ irradiance, there were no significant differences among treatments, but all treatments were significantly different from controls. These data indicate that GA-treated cells, like K-treated ones, entered exponential growth sooner than control cells. Adair and Miller (1982) reported a similar response for GA-treated *Cyclotella cryptica*.

Significant differences due to IAA treatment of *Chlorella* occurred only for those treated with 10 $\mu g \cdot ml^{-1}$. Grotbeck and Vance (1972) showed that the endogenous level of IAA in synchronized *Chlorella* cells doubled just prior to cell division. This finding, coupled with the data of Peterson's (1972) that nuclear DNA and RNA were correlated with exogenous IAA, leads one to suspect a regulatory role.

Experiments using all possible combinations of the three phytohormones revealed no additive, synergistic, or antagonistic effects in *Chlorella*. Since results were comparable to those obtained with individual hormones, they are not presented.

The mere occurrence of phytohormones in algae is not evidence that such compounds function as growth regulators in vivo. Proof of a metabolic role would require evidence that the growth and/or differentiation and development of the algae would be impaired in the absence of phytohormone. Such data have not yet been conclusively obtained and reported (Buggeln, 1976). Nevertheless, the significant reduction in time to incipient cell division in *Chlorella* to hormonal concentrations of K, GA, and, to a lesser extent, IAA indicates a growth-regulating role for these phytohormones.

References

Adhikary SP, Pattnaik H (1978) Effects of hormones on growth of *Westiellopsis prolifica* Janet, a blue-green alga under light and dark treatments. Indian J Exp Biol 16:1212–1213

Adair OV, Miller MW (1982) Growth responses of the diatom, *Cyclotella cryptica* (Bacillariophyceae), to gibberellic acid. J Phycol 18:587–589

Ahmad MR (1973) The effect of gibberellic acid, kinetin and indole-3-acetic acid on the growth of blue-green algae in culture. Rev Algol 11:154–160

Arendarchuk VV (1978) Indole containing substances of some blue-green algae. Gidrobiol ZH (USSR) 14:78–80.

Berliner MD (1981) Hormone effects on *Cosmarium botrytis* cell division. Cytobios 30:89–100

Bralczyk J, Wielgat D, Wasilewska-Dabrowska LD, Kleczkowski K (1978) Growth accelerating response of *Euglena gracilis* Z. to gibberellic acid. Plant Sci Lett 12:265–272

Buggeln RG (1976) Auxin, an endogenous regulator of growth in algae? J Phycol 12:355–358

Buggeln RG, Craigie JS (1971) Evaluation of the evidence of the presence of indole-3-acetic acid in marine algae. Planta 97:173–178

Chapman RW, Vance BD (1976) Ethephon (2-chloroethylphosphonic acid) effects on *Scenedesmus quadricauda*. J Phycol 12:146–149

Conrad HM, Saltman P (1962) Growth substances. In: Lewin RA (ed) Physiology and biochemistry of algae. Academic Press, New York, pp 663–671

Czerpak R (1979) Effect of some natural and artificial growth regulators on the chemical composition of autotrophic miroorganisms *Anabaena variabilis, Chlorella pyrenoidosa* and *Scenedesmus acumintus*. Bull Acad Pol Sci Soc Sci Biol 2:591–604.

Grotbeck L, Vance BD (1972) Endogenous levels of indole-3-acetic acid in synchronous cultures of *Chlorella pyrenoidosa*. J Phycol 8:272–275

Horgan R (1984) In: Wilkins MB (ed) Advanced plant physiology. Pittman, London pp 53–75

Huang TC, Chow TJ (1984) Ethylene production by blue-green algae. Bot Bull Acad Sci 25:31–86.

Ishiura M (1976) Gametogenesis of *Chlamydomonas* in the dark. Plant Cell Physiol 17:1141–1150

Jennings RC (1969) Cytokins as endogenous regulators in the algae *Ecklonia* (Phaeophyta) and *Hypnea* (Rhodophyta). Aust J Biol Sci 22:621–627

Kim WK, Greulach VA (1961) Promotion of algal growth by IA, GA and kinetin. Plant Physiol 36(Suppl 14):45

Leonard NS, Hecht SM, Skoog FS, Schnitz RY (1968) Cytokinins: Synthesis of 6(3-methyl-1-3-butenylamino)-9-8-0-ribofuranosylpurine and the effect of side-chain unsaturates on the biological activity of isopartylaminopurines and their ribosides. Proc Natl Acad Sci USA 59:15–21

Monoson H, Fried R, Galsky A (1979) The induction of auto colony formation in *Scenedesmus quadricauda* by auxins and low pH. Plant Physiol 63(Suppl 5):21

Mowat JA (1965) Survey of the results on the occurrence of auxins and gibberellins in algae. Bot Marina 8:149–155

Peterson JA (1972) Effect of indole-3-acetic acid on the nucleic acids of synchronous cultures of *Chlorella pyrenoidosa*. M.A. Thesis (unpublished), North Texas State University

Provasoli L, Carlucci AF (1974) Vitamins and growth regulators. In: Stewart WDP (ed) Algal Physiology and Biochemistry. University of California Press, Berkeley, pp 741–787

Sorokin C, Krauss RW (1965) The dependence of cell division in *Chlorella* on temperature and light intensity. Am J Bot 52:331–339

Swaminathan S, Bock RM (1977) Isolation and identification of cytokinins from *Euglena gracilis* var—*bacillaris* transfer RNA. Biochem 16:1355–1360

Tatkowska E, Buczek J (1980) Effect of phytohormones on the growth of *Scenedesmus quadricauda*. Acta Soc Bot Pol 49:211–220

Taylor IEP, Wilkinson AJ (1977) The occurrence of gibberellins and gibberellin-like substance in algae. Phycologia 16:37–42

Wood NL, Berliner MD (1979) Effects of IAA on the desmid *Micrasterias thomasiana*. Plant Sci Lett 16:285–290