

BRIEF COMMUNICATION

Effect of red radiation, kinetin and linuron on growth and ethylene production in *Chlorella*

V.D. KRESLAVSKY, E.F. KOBZAR' and E.N. MUZAFAROV

*Institute of Soil Sciences and Photosynthesis RAS, Pushchino, Moscow Region, 142292 Russia***Abstract**

Both short-term and continuous red radiation stimulated while far red radiation inhibited growth and ethylene production in *Chlorella pyrenoidosa*. Kinetin and linuron also affected culture density and ethylene production, depending on their concentration. Phytochrome might participated in the regulation of growth and ethylene production.

Additional key words: algae, phytochrome.

Synthesis of ethylene (C_2H_4) in higher plants is regulated by red (R) and far red radiation (FR) (Vangronsveld *et al.* 1988, Lieberman 1979), this process being phytochrome-dependent. Also there are data about influence of exogenous cytokinins, including derivatives of phenylurea (linuron) and kinetin, on ethylene production (Abeles 1973, Kulaeva 1973, Lieberman 1979, Romanovskaya 1985, Ishisawa and Esashi 1983). Analogous study in unicellular algae which produce ethylene in large amounts (Osborne 1978), may be of interest. The aim of this work was to compare light and hormonal regulation of ethylene production in microalga *Chlorella pyrenoidosa*. When studying hormonal regulation, we paid attention to the action of cytokinins, which are known to be present in microalgae and to stimulate their growth (Osborne 1978).

The culture of green alga *Chlorella pyrenoidosa* Pringch 82T was grown on the medium of Tamiya at temperature 32 - 36 °C (for details see Vladimirova and Semenenko 1962). Suspensions were aerated with an air + 2 % CO_2 gas mixture. Control and experimental cuvettes were irradiated with green radiation (40 W m^{-2} , $\lambda_{\text{max}} = 530\text{ nm}$). Red (R, $0.25 - 1.00\text{ W m}^{-2}$, $\lambda_{\text{max}} = 660\text{ nm}$) and far red (FR, $2 - 5\text{ W m}^{-2}$, $\lambda_{\text{max}} = 730\text{ nm}$) radiations were given in addition to the background green radiation.

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Kinetin and linuron were used at concentrations of 10^{-10} - 10^{-4} M. The number of cells and dry mass were measured after 2 - 3 d. Ethylene production was determined by gas chromatograph *Tsvet-101* on the 3rd day of growth, when culture density (n) reached $50 - 100 \times 10^6$ cells dm^{-3} . The rate of photosynthesis was measured as the rate of O_2 -evolution with a Clark-type oxygen electrode. Experiments on the effect of R, kinetin and linuron on culture growth and C_2H_4 -production were replicated 7 - 9 times and experiments with FR 5 times.

Supplementary continuous R irradiation (24 h) stimulated the culture growth by 10 - 20 % as compared with control, FR abolished this effect. Cell size in these experiments did not change significantly (change < 3 %). Rates of photosynthesis measured in the control (culture grown under green radiation only) and in experiments with R and FR did not differ significantly.

Similarly, long-term R irradiation (12 - 18 h) stimulated ethylene production (Fig. 1). This stimulatory effect reached maximum in the 6 h and then gradually lowered to the initial level. On the other hand, long-term FR itself inhibited ethylene production (Fig. 1). During short-time exposure (10 - 30 min) the effect of R stimulation also took place, being nullified by following FR, demonstrating R/FR reversibility.

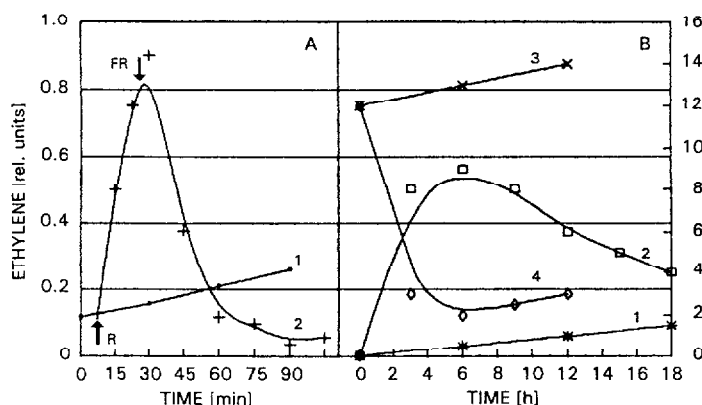


Fig. 1. Production of ethylene by *Chlorella* as affected by supplemented R/FR irradiance: A - short-term (10 - 30 min) R and FR effect (1 - control, $n_0 = 70.5 \times 10^6$ cell dm^{-3} ; 2 - experiment with R/FR) and B - long-term (12 - 18 h) R and FR effect (1,2 - control and experiment with R, $n_0 = 77 \times 10^6$ cell dm^{-3} ; 3,4 - control and experiment with FR, $n_0 = 180 \times 10^6$ cell dm^{-3}).

The possibility of regulation of different processes in microalgae by R/FR is of great importance, because it is connected with photoreceptor functioning in cells. The question about availability of phytochrome in microalgae is still open. Some published results favour the presence of phytochrome in microalgae, *e.g.*, Ruyters (1988) discovered R/FR regulation of respiration in green unicellular alga *Dunaliella*. Our results demonstrating that R and FR influence ethylene production in opposite way in *Chlorella* are in agreement with the idea of phytochrome regulatory action. Similarly, R stimulates growth of *Chlorella*, FR itself inhibits it and also reverses the

stimulatory effect of R. This photoregulation of culture growth seems to be also controlled by phytohormone. It is likely that many other physiological functions and responses in green algae may also be controlled by phytochrome.

We studied kinetics and concentration dependence of *Chlorella* growth in the presence of kinetin and linuron. Maximum density of suspension was observed after 15 h for kinetin and 24 h for linuron. The maximum stimulation of growth by kinetin (25 %) was observed at the concentration of 10^{-8} M and by linuron (35 %) at the concentration of 10^{-9} M (Table 1). Concentrations of kinetin $>10^{-6}$ M and linuron $>10^{-7}$ M led to growth inhibition.

Also ethylene production depended on kinetin and linuron concentration. We found a considerable increase in ethylene production at kinetin concentration of 10^{-6} - 10^{-7} M, while at concentration of 10^{-5} - 10^{-4} M inhibition of the process was observed (Table 1). Linuron stimulated ethylene production at concentration of 10^{-9} - 10^{-7} M (Table 1). Maximum stimulation (100 %) occurred at the concentration of 10^{-9} M. Stimulatory effects had a long-term character, reaching their maximum after 24 - 28 h. Concentrations of linuron $>10^{-7}$ M inhibited ethylene production.

Dependence of ethylene production and culture density on concentration of linuron and kinetin (control without linuron or kinetin = 1; n_0 = 104.5 measured 24 h after addition of linuron and n_0 = 127.0 measured 15 h after addition of kinetin).

Linuron concentration [M]	C ₂ H ₄ [rel. units]	culture density [rel. units]	Kinetin concentration [M]	C ₂ H ₄ [rel. units]	culture density [rel. units]
10^{-6}	0.2 ± 0.007		10^{-5}	0.5 ± 0.010	0.88 ± 0.009
3×10^{-7}	0.8 ± 0.028	0.72 ± 0.007	10^{-7}	1.4 ± 0.040	1.19 ± 0.013
10^{-7}	1.1 ± 0.035	1.00 ± 0.012	10^{-8}	1.8 ± 0.056	1.25 ± 0.014
10^{-8}	1.5 ± 0.049	1.23 ± 0.014	10^{-10}	1.1 ± 0.035	1.00 ± 0.012
10^{-9}	1.9 ± 0.060	1.35 ± 0.016			
10^{-10}	1.0 ± 0.035	1.10 ± 0.012			

Thus, literature and our experimental data lead to the conclusion that biosynthesis of ethylene and growth of green unicellular algae may be regulated by red radiation and growth stimulatory substances with cytokinin activity.

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