Influence of Growth Regulators Kinetin and 2,4-D on the Growth of two Chlorophyte Microalgae, *Haematococcus pluvialis* and *Dunaliella salina*

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ABSTRACT

Both *H. pluvialis* and *D. salina* are very interesting *Chlorophyte* microalgae but have slow growth rates. Taking this into account, and knowing the effects hormones have on plants, two growth regulators, in different combinations, were tested and final concentration of microalgae evaluated.
***Dunaliella salina* showed very good results with all the hormone combinations used when grown under 15% salinity, except for 0.5mg auxin 2,4-D/l and no kinetin; under 10% salinity growth was best with 0.5mg auxin 2,4-D/l and no cytokinin kinetin and also very good with 1.0mg 2,4-D/l and no kinetin , and 2.0mg 2,4-D and 0.5mg kinetin/l of culture medium.**
The best growth for *Haematococcus pluvialis* was obtained with 1.0mg 2,4-D/l medium, but also showed a very good growth rate when the ratio auxin:cytokinin equalled 1 (better with 1.0mg of both hormones), and under 0.5mg 2,4-D and 2.0mg kinetin/l of culture medium.

INTRODUCTION

***Haematococcus pluvialis* and *Dunaliella salina* are two of the most cultivated unicellular, biflagellated, microalgae. *H. pluvialis* is a freshwater microalga and *D. salina* can tolerate salinities up to 35% NaCl (w:v) (Ben-Amotz & Avron 1990), but both of them are chlorophytes and produce high amounts of carotenoids (Ben-Amotz & Avron 1990, Borowitzka 1992, Lee & Ding 1994) .**
However, because of their slow growth rate, several methods were used to enhance the productivity in a first, pre-carotenogenic phase.
It is known that, in higher plants, auxins usually act as promoting the elongation of cells and, with cytokinins, stimulate cellular division (Aloni 1990, Cleland 1990, Skoog & Schmitz 1972); these last hormones also promote chloroplast development. But, most of the times it is the balance of hormones present in a plant cell that produces the effect, more than the presence or absence of one particular hormone (Mader 1985).
Since, in the first pre-carotenogenic phase the main objective is the production of more biomass in less time than the usual, we proposed to study what could be the influence of adding two plant hormones, or growth regulators, to the culture media of *Dunaliella salina* and *Haematococcus pluvialis*, and evaluate the corresponding increase in cell number. The other reason that motivated us to this study was the fact that there is almost nothing published on this subject.

MATERIAL AND METHODS

Microalgae

***Haematococcus pluvialis* 34/7 CCAP strain; *Dunaliella salina* 10/30 CCAP strain.**

Culture media

For *H. pluvialis*, Bold (1949) modified: urea 1.5mM was used instead of sodium nitrate, pH 7.02-7.38. For *D. salina*, Johnson's medium (Johnson *et al.* 1968) modified: 1.0g potassium nitrate per litre medium was used, and desired salinities (10% and 15 %, w/v) were obtained by adding 100g or 150g sodium chloride per litre, pH 8±0.1.

Growth regulators

Growth regulators (kinetin and 2,4-D) were prepared as solutions in NaOH 1M, 1mg hormone/ml solution. Final concentrations in the cultures were 0.5, 1.0 and 2.0mg/l. Sixteen combinations were tested (table 1), the control (A) having neither kinetin or 2.4-D.

Table 1. Distribution of different hormone concentrations in cultures

		kinetin (mg/l culture medium)			
		0.0	0.5	1.0	2.0
2,4-D (mg/l culture medium)	0.0	A	B	C	D
	0.5	E	F	G	H
	1.0	I	J	K	L
	2.0	M	N	O	P

Algal cultivation

Experiments were carried out in tubes, with 40ml culture, either for *Haematococcus* and *Dunaliella*, under constant temperature (21º C) and light (white fluorescent tubes, 62µmol photons $m^{-2}s^{-1}$). Triplicates were always made for all the experiments and results represent the average.
Growth was determined by cell countings, which were made using a Neubauer improved chamber.

Statistical analysis

***Statistica* package was used to evaluate the significance between the results obtained for the different hormone combinations. Means were compared by Tukey's HSD test ($p<0.05$). Values analysed were the growth ratios Gi/G0, being Gi the growth along time and G0 the initial number of cells.**

ACKOWLEDGMENTS

The authors would like to thank Maria João Barbosa, Ana M. Guadalpi, Dora Santos and Gonçalo Tavares for their grateful help on doing this piece of work.

RESULTS AND DISCUSSION

Some hormone combinations actually promoted a significant growth increase on the cultures either of *H. pluvialis* or *D. salina*, this enhancement being most obvious when *Dunaliella* grew under 15% salinity (NaCl, w:v). At this salinity (first figure), only the concentration/ combination 0.5mg 2,4-D and no kinetin resulted in a inhibition of growth.

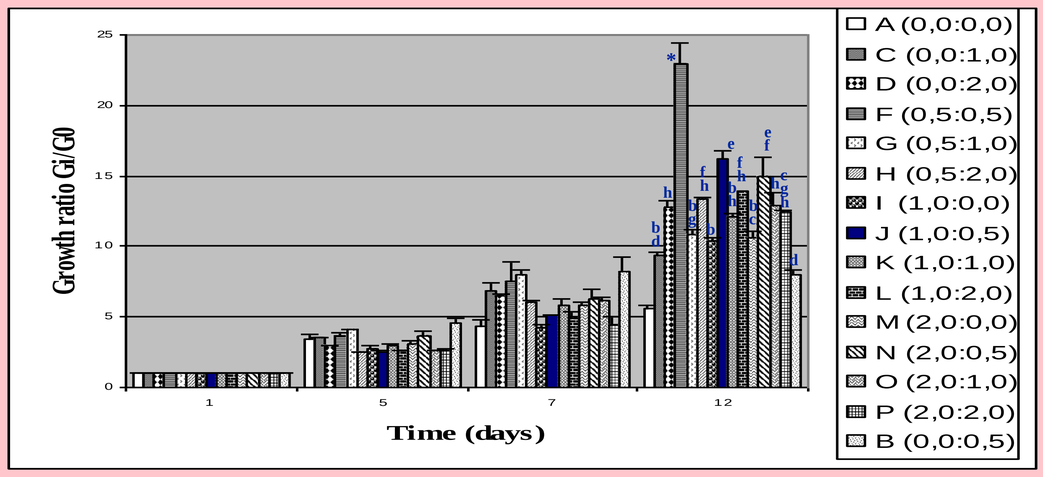

Figure 1. The graphic represents the growth ratio (Gi/G0) for *Dunaliella salina* under 15% salinity (NaCl, w/v).

For both *Haematococcus* (third figure) and *Dunaliella* (under 10% salinity, second figure) only three combinations induced a significant increase on the growth ratio. Nevertheless, most of the hormone combinations showed no significant effect on the growth of these microalgae, or even resulted in a inhibition of the cultures development.

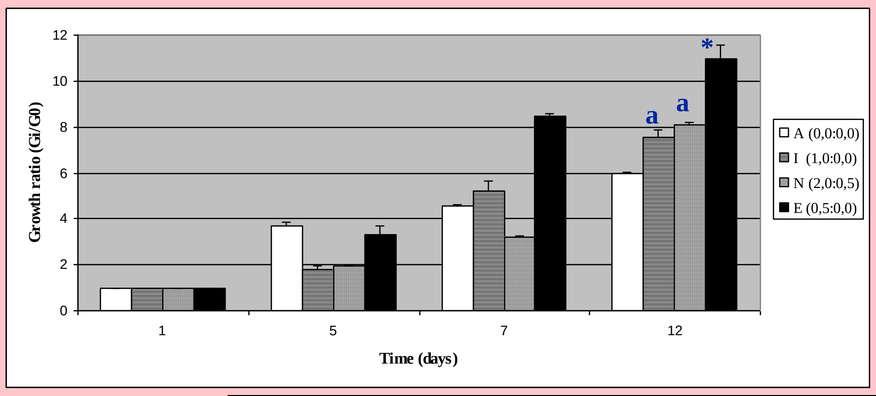

Figure 2. The graphic represents the growth ratio (Gi/G0) for *Dunaliella salina* under 10% salinity (NaCl, w/v).

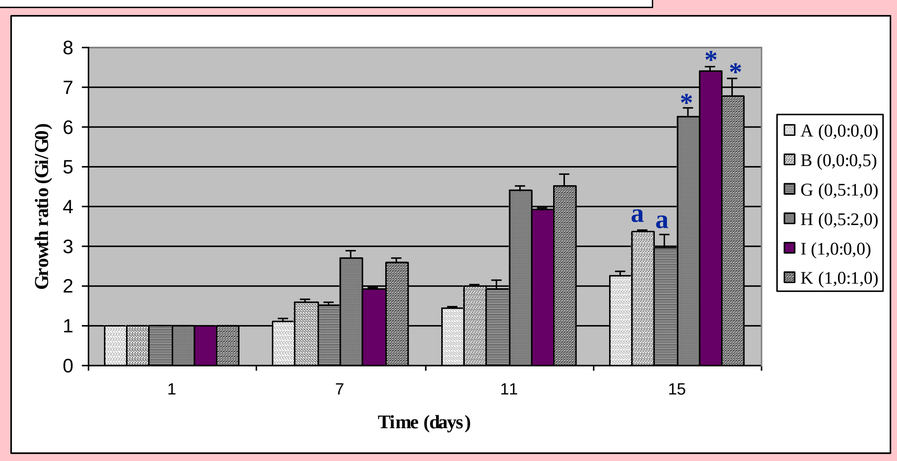

Figure 3. The graphic represents the growth ratio (Gi/G0) for *Haematococcus pluvialis.*

On the figures, same letter indicates growth under different conditions (combinations of growth regulators) is not significantly different ($p>0.05$).
(*) indicates samples are significantly different from control (condition A) and all other conditions ($p<0.05$).
Values represented are only the ones which are significantly different from the control (condition A), without any growth regulator.
The biggest difference, and most obvious, happened with *Dunaliella salina*, under 15%salinity. This difference in growth might be related either to concentration of effective hormone or cell sensitivity, created by the increase in salinity. Salinity could also had had some influence on the uptake efficiency of the exogenously applied hormones, modifying in some way the sensitivity of cells. Moreover, as the maintenance of rapid cell enlargement and division requires some solute that can be absorbed (Stevenson & Cleland 1981), NaCl might be responsible for the higher growth rates at 15%S, as it is one of the effective solutes, at optimal concentration.

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