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EFFECT OF DIFFERENT LIGHT INTENSITIES ON *HAEMATOCOCCUS PLUVIALIS* KETOCAROTENOID BIOSYNTHESIS

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Haematococcus pluvialis is a freshwater unicellular alga able to accumulate large amounts of ketocarotenoids, in particular astaxanthin, in response to various stress conditions, such as high light, salinity, acetate addition, nutrient stress and high carbon/nitrogen ratio.

This compound with strong antioxidant properties is widely used for nutraceutical and pharmaceutical purposes.

The aim of this work was to investigate ketocarotenoid biosynthesis in *H. pluvialis* by evaluating the physiological responses of this alga to different light conditions (dark, 20 μmol m^{-2} s^{-1} and 80 μmol m^{-2} s^{-1} continuous light).

Biomass yield, phenotypic changes, pigment accumulation and transcriptional expression levels of five genes involved in the ketocarotenoid biosynthetic pathway (*isopentenyl pyrophosphate isomerase IPI, phytoene synthase PSY, lycopene β-cyclase LYC, β-carotenoid oxygenase CRTO,* and *carotenoid hydroxylase CRT-B*) were evaluated. All analyses were performed at day 3 and 7 of the light treatment and algal cultures kept in the dark were used as a control.

In general, quantitative real time PCR analyses revealed that a down or a no significant regulation of the five ketocarotenoid biosynthetic genes occurred on day 3, whereas an up-regulation was observed on day 7 at both 20 and 80 μmol m^{-2} s^{-1}.

In particular, *IPI* and *PSY*, coding for enzymes catalysing the upstream reactions in the carotenoid pathway were upregulated at 20 μmol m^{-2} s^{-1}, whereas no significant differences compared to control were observed at 80 μmol m^{-2} s^{-1}.

LYC, the gene coding for lycopene synthase, the enzyme responsible for the cyclization of lycopene into β-carotene, and *CRTO* and *CRTR*-B, the two genes involved in the last steps of astaxanthin production, showed an up-regulation at both light conditions. *LYC* and *CRTO* showed no significant differences in expression between 20 and 80 μmol m^{-2} s^{-1}, whereas *CRTR-B* was upregulated at higher level at 20 μmol m^{-2} s^{-1}.

With regard to carotenoid accumulation, the maximum amount of these compounds was detected on day 7 at 80 μmol m^{-2} s^{-1}, although an increase in pigment content was already evident on day 3 at the same light condition.

In conclusion, the lack of direct correlation between ketocarotenoid biosynthetic gene expression and pigment accumulation, suggests the presence of other regulatory mechanisms involved in the biosynthesis of these compounds.

Further studies are necessary to understand the complex mechanism underlying ketocarotenoid biosynthesis in *H. pluvialis* in order to optimize culture parameters and astaxanthin large-scale production.