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NUCLEAR TRANSFORMATION OF TWO CELL WALL-LESS STRAINS OF *CHLAMYDOMONAS REINHARDTII* FOR THE OVEREXPRESSION OF TWO PHYTOENE SYNTHASE EXOGENOUS GENES

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Microalgae have remarkable metabolic plasticity, easy and rapid growth capacity, which have induced the researches to focus on possible bioaccumulation abilities of highly productive strains, also considering the opportunities offered by genetic transformation. In microalgae, the chloroplast can represent an interesting compartment for the accumulation of molecules of interest, such as carotenoids ("cell factory"). The aim of this work was the genetic nuclear transformation of the green microalga *Chlamydomonas reinhardtii* cw-less strains in order to obtain carotenoid accumulation inside the chloroplast, through the expression of a key-enzyme involved in the carotenoid biosynthetic pathway, the phytoene synthase (PSY).

Two different expression vectors were obtained via Gateway Technology recombination and used for the nuclear transformation of cc-3491 and cc-400 cw-less strains, using glass beads method:

- pPSYB: β-tubulin promoter + intron *rbcs2* + cDNA enconing for *AtPSY* + *rbcs2* terminator + *aph7''*
- pPSYD: β-tubulin promoter + intron *rbcs2* + cDNA enconing for *OsPSY1* + *rbcs2* terminator + *aph7''*

After the transformation, Hygromycin-resistant colonies appeared for both strains, but a higher and more stable transformation efficiency was obtained for cc-3491, with a percentage of Hygro-PSY positive colonies of 60% for pPSYB and 100% for pPSYD Hygro-resistant colonies.

At present, a yellowish phenotype has been observed in only one transformant, B3, deriving from cc-3491 transformed with pPSYB, grown in liquid medium for two months. The photosynthetic pigment analysis revealed the same amount of chlorophylls, but a higher content of carotenoids (+40%) with respect to the control. New inocula from this culture were monitored at 5, 14 and 30 days after the inocula, showing at the 30th day a higher level of pigments with respect to the control (+62% chlorophylls, +25% carotenoids). Moreover, the PAM fluorimetry of B3 revealed a lower proportion of photoinactivated PSII after 5 min of high light exposure (1400 μmol m^{-2} s^{-1}), indicating a more effective photoprotection to high light. This is probably due to a different quantity and/or quality of carotenoids. Biochemical analyses are planned in order to confirm the presence of

the exogenous PSY protein in B3, and HPLC analysis will be performed in order to determine the composition of carotenoids.