

## GENOME-EDITED *N. BENTHAMIANA* PLANTS ACCUMULATING ZEAXANTHIN FOR THE PRODUCTION OF SAFFRON CROCINS

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*Nicotiana benthamiana* is a model system for plant transformation and is used as a platform for the production of biopharmaceuticals and small molecules. Here we describe a transient and stable combinatorial genome editing method, followed by direct LC-MS screening for zeaxanthin accumulation, for the genome editing of the *Lycopene Epsilon Cyclase* and the *Zeaxanthin Epoxidase* genes. Our method allows to obtain Cas9-free T2 plants accumulating zeaxanthin, up to 350.3 µg g<sup>-1</sup> of Dry Weight (DW), as the only leaf xanthophyll. In face of several alteration of edited *N. benthamiana* plants for photosynthetic parameters, such as the compensatory increase in PSII/PSI ratios and a loss of the large-size PSII supercomplexes, results confirmed the antioxidant and photoprotective role of zeaxanthin in vivo. In addition, two independent High-Zeaxanthin lines (HZ), triple *lcy1 lcy2 zep2* (HZ-9) and quadruple *lcy1 lcy2 zep1 zep2* (HZ-11) K0 plants, were efficiently used as a chassis for the production of crocins (apocarotenoid glucosides), zeaxanthin cleavage products normally accumulated in saffron, with anti-oxidant, anticancer, and neuro-protective properties. Two different methods of transient overexpression of the saffron *CsCCD2*, catalyzing the first dedicated step in crocin biosynthesis,

were compared: i) agroinfiltration with *Agrobacterium tumefaciens* and ii) infection with the viral vector derived from tobacco etch virus (TEV). Following viral infection we observed the higher levels of crocins, up to 1129 µg/g dry weight, and comparing results even with WT plants, we observed that while in vitro and in bacterio *CsCCD2* was described to strongly prefer zeaxanthin, in planta there is a relaxed substrate specificity of *CsCCD2*, which is able to cleave also lutein and, to a lesser extent, violaxanthin and β-carotene (Demurtas et al 2023).

## Reference

Demurtas, O. C., Sulli, M., Ferrante, P., Mini, P., Martí, M., Aragonés, V., Aragonès, Ja Daròs & Giuliano, G. (2023). Production of Saffron Apocarotenoids in *Nicotiana benthamiana* Plants Genome-Edited to Accumulate Zeaxanthin Precursor. *Metabolites*, 13(6), 729.