

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

SULLI M.\*, DEMURTAS O. C.\*, FERRANTE P.\*, DALL'OSTO L.\*\*\*, GUARDINI Z.\*\*\*,  
MINI P.\*, APREA G.\*, NICOLIA A.\*\*\*, MARTÍ M.\*\*\*\*, ARAGONÈS V.\*\*\*\*,  
BASSI R.\*\*\*, DARÒS J.\*\*\*\*, GIULIANO G.\*

\*) Italian National Agency for New technologies, Energy and Sustainable Development (ENEA), Casaccia Res Ctr, Via Anguillarese 301, 00123 Roma Italy

\*\*) University of Verona, Via Ca' Vignal 1, 37134, Verona, Italy

\*\*\*) Council for Agricultural Research and Economics, Research Centre for Vegetable and Ornamental Crops, Via Cavalleggeri 25, 84098 Pontecagnano, Italy

\*\*\*\*) IBMCP (CSIC-UPV), Avenida de los Naranjos s/n 46022 Valencia, Spain

*zeaxanthin, crocins, Nicotiana benthamiana, New Plant Breeding Techniques (NPBTs), photosynthesis*

*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.