Proceedings of the LXIV SIGA Annual Congress
Online, 14/16 September, 2021
ISBN: **978-88-944843-2-8**

Oral Communication Abstract – 6.05

METABOLIC ENGINEERING OF CURCUMINOIDS IN N. BENTHAMIANA LEAVES

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Curcuminoids, metabolic engineering, plant

Curcuma longa is a tropical plant belonging to the family of Zingiberaceae. Commonly called turmeric or curcuma, it has been used for thousand years as a yellow and aromatic spice and the powder is largely used in curry mixtures. Its color is due to the presence of curcuminoids, a family of active compounds of the phenylpropanoid group which, due to their therapeutic properties, have given a strong interest to this spice in recent years. Curcumin and its analogs have been shown to act as strong antioxidants and are involved in a broad range of diseases prevention. Moreover, curcumin presents a strong anticancer activity against melanoma, endometrial carcinoma, glioma and breast cancer. Unfortunately, curcuminoids are characterized by a low bioavailability which limit their bioactivity. Notably, it is also known that their association with lipid-like molecules, like carotenoids and apocarotenoids, increases the absorption of curcumin itself without altering its antioxidant and anticancer activities.

Genes responsible for curcuminoids synthesis have been previously identified and heterologous expression in microbial systems have been carried out, resulting in successful accumulation in curcumin and curcumin-related compounds. On the contrary, no efforts of metabolic engineering of curcuminoids in plant heterologous systems have been reported to date.

In this context, the aim of this study was to verify if moving curcuminoids biosynthetic pathway in the plant model system *Nicotiana Benthamiana* L. may lead to the accumulation of these metabolites.

Since principal metabolic reactions in the curcuminoids pathway start downstream phenylpropanoid precursors accumulation, we focused our attention in two enzymatic functions: the diketide-CoA synthase (*DCS1* and *DCS2*) and the curcumin synthase (*CURS1* and *CURS3*) genes. *N. benthiamana* leaves were then transiently transformed using *DCS* and *CURS* genes in different combinations, with a consequent accumulation of curcuminoids in less than 2 weeks. Interestingly, in all cases, curcumin was the most abundant metabolite, followed by demethoxycurcumin and bisdemethoxycurcumin respectively, and the combination *DCS1+DCS3+CURS1+CURS3* was the one showing the highest curcumin level.

Finally, we simultaneous transformed *N. benthamiana* leaves with four plamids yielding a series of well-known transcription factors (TFs) boosting the accumulation of different phenylpropanoid classes (flavonoids, anthocyanins etc), in order to verify the effectiveness of any of them to promote the curcuminoids branch: within all tested conditions, only the plasmid carrying *Rosea* and *Delila* TFs was able to achieve a ≈ 2.15 fold curcumin accumulation compared to the *DCS+CURS* construct alone. Overall, this study represents the first attempt in the direction of the stable metabolic engineering of curcuminoids in plant systems.