

CYNARA CARDUNCULUS CALLUS CULTURES AS BIOFACTORIES: A PROMISING APPROACH TOWARDS SUSTAINABLE PRODUCTION OF MONOUNSATURATED FATTY ACIDS

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The development of effective tools for sustainable supply of phyto-ingredients and natural substances with reduced environmental footprints in substitution of non-renewable materials can help mitigating the dramatic scenario of climate changes and global warming. The use of plant cell cultures as sustainable biorefineries can be a technological advancement to face this challenge and offer a potentially unlimited availability of natural substances, in a standardized composition and devoid of seasonal variability compared to field-cultivated plants. Cardoon (*Cynara cardunculus* L. var. *atilis*) callus cultures can produce fatty acids and accumulate polyphenols, being therefore suitable for large-scale production of biofuel precursors, as well as biochemicals and valuable compounds.

Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), besides their value in food and nutrition, are attracting considerable attention as supplements in the formation of biodegradable plastics, bio-additives for cosmetic industry and bio-lubricants. With the aim of boosting their potential uses, we designed a biotechnological approach in cardoon callus cultures to increase oleic acid content through an *Agrobacterium tumefaciens*-mediated metabolic engineering strategy. Bioinformatic data mining in the *C. cardunculus* transcriptome (www.artichokegenome.unito.it) allowed the

selection of *SAD* (Stearic acid desaturase) and *FAD2.2* (fatty acid desaturase) genes and their further molecular characterization. A *SAD* gene coding for the key desaturase enzyme in oleic acid formation was used for gene overexpression, and a *FAD2.2* gene encoding a desaturase enzyme involved in linoleic acid formation was used for RNAi silencing. Here, we report the molecular and metabolic characterization of *SAD*-overexpressing and *FAD*-silenced callus lines. Successfully, both engineered lines produced higher content of oleic acid compared to wild type calli. Notably, overexpressing *SAD* callus lines showed an increase in oleic acid content along with a rise in the entire metabolic flux, whereas *FAD2.2* silenced lines showed a distinct linoleic acid reduction in favor of accumulation of its precursor, oleic acid. Testing of the produced transgenic lines at a pilot-scale will validate the effectiveness of our research approach for the creation of a cardoon cells-based biorefinery. Besides, the use of industrial and/or agricultural waste and by-products as components of nutritive substrates could improve the sustainability of the process, contributing to the set-up of a sustainable, innovative biotechnological approach to provide valuable supplies for biocosmetics and green chemistry, according to the principles of Circular Economy.