Investigadores: **Antonio Granell, Clara Pons**
Proyecto: **Engineering strategies for functional characterization of tomato "good" flavour alleles**

Tomato (*Solanum lycopersicum*) is one of the world's most widely consumed fruits, valued for its culinary uses, nutritional content, and characteristic flavour. Tomato contain a wide array of compounds, some of them such sugars, organic acids, polyphenols and volatile compounds (VOCs) play a pivotal role in creating the distinctive taste and aroma. The balance and interaction of these compounds determine the overall flavour of tomatoes. In the last 40 years, however, a growing number of consumers express dissatisfaction with the flavour of modern commercial tomatoes. This decline in flavour is largely the result of modern breeding as well harvesting and post-harvest handling techniques, which have prioritized traits important to growers and distributors—such as productivity, disease resistance, shelf life, and external appearance—neglecting qualities that consumers value, such as flavour and nutritional quality. Over the last thirty years, emphasis has increased on identifying genetic and molecular factors responsible for traits like fruit size, shape, ripening, flavour, and nutritional composition, all of which are crucial for addressing both consumer and agronomic priorities.

The development of varieties with improved traits, such as increased brix and/or favourable VOCs, requires the use of natural variations and the identification of stable genetic segments carrying traits of interest. In a previous research in our lab we have identified an introgression line (IL9-2) derived from *S. pimpinellifolium* cv TO397 x *S. lycopersicum* cv Moneymaker (MM) cross that significantly qualified as better tasting than MM in sensory analyses and contains a region of ~95 Kb that increased the levels of ºBrix (sugars) and the VOC benzylalcohol, associated to floral, fruity and sweet flavour. Within this region, there are ten annotated genes among with Lycopersicum Invertase5 (LIN5), which encodes a cell wall invertase, controls total soluble solids content in tomato, and a NAD(P)-dependent SDR alcohol dehydrogenase are the most promising candidate genes for Brix and benzyl alcohol, respectively. In previous studies, using a *S. pennellii* IL derived population, revealed that the wild species allele of LIN5 was more efficient than the cultivated allele, due to a single nucleotide substitution that coded for an amino acid residue close to the near the catalytic site of the invertase crystal, affecting enzyme kinetics and fruit sink strength. To further understand molecular and biochemical basis of the changes in Brix and benzyl alcohol produced by TO397 alleles in the IL9-2, in this project we propose the heterologous overexpression of TO397 LIN5 and NAD(P)-dependent SDR alcohol dehydrogenase proteins in the wine yeast *Saccharomyces cerevisiae* to assay both Invertase activity and benzyl alcohol dehydrogenase activities. Results of these experiment will reveal details on the specificity and kinetic properties of the enzymes that may explain the observed phenotypes in the fruit. In addition docking studies will be done using protein modelling Alphaphold and Rosetta that will allows to understand those differences at the molecular /mechanicistic level

During the course of the project the student will learn multiple techniques of molecular biology, biochemistry and biotechnology techniques

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