CRISPR/Cas9-mediated genome editing to improve the nutritional quality of tomato fruit

Aurelia Scarano[1], Eugenio Butelli[2], Cathie Martin[2] and Angelo Santino[1]

[1]National Research Council, Institute of Science of Food Production (CNR-ISPA), Lecce, Italy, [2]John Innes Centre, Norwich, UK

Metabolic engineering in tomato has been successfully applied for its nutritional improvement, since tomato is an excellent candidate for the genetic engineering/manipulation, by using either the conventional transgenesis, or the novel approach of genome editing (GE).

CRISPR/Cas9-mediated GE is emerging as a modern technology in plant research field, especially for the possible introduction of genetic traits, important for agronomy and nutritional quality of fruits, vegetables and staple crops. Site-specific, precise modifications can be addressed on the target gene, using the CRISPR/Cas9 system associated with RNA guide/s (sgRNA). The double strand breaks introduced by Cas9, can be repaired through the endogenous systems of repair (NHEJ and HR), creating insertions/deletions (indels). The NHEJ (non-homologous end joining) system re-join imprecisely the separated ends, while the HR (homologous recombination) system uses a donor template to repair the break. To date, few studies have been carried out using the HR system of repair in tomato. In some cases, it is necessary to use molecular strategies to increase the copy number of the donor template in the cell, in order to increase the probability of the targeting events. Among them, the use of viral sequences (i.e. LIR and RepLIR from BeYV), inducing a rolling circle replication, can be a valid approach. We aimed to introduce the whole sequence of the fruit-specific promoter E8 upstream the MYB12 gene, involved in the enhancement of polyphenol biosynthesis in tomato fruit. We identified one correct gene targeting event and 3 partial insertions out of 60 hairy roots samples, in a transient expression assay mediated by *Agrobacterium rhizogenes*. Furthermore, in our hands the presence of the viral sequences LIR and RepLIR, also induced molecular re-arrangements in the plasmid backbone, during the *A.tumefaciens*-mediated transformations. Such re-arrangements affected plasmid stability and were bacterial strain-dependent. In this context, the HR remains challenging, although it would be a novel way to engineer the metabolic pathways for tomato nutritional improvement.