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expression, we employed a CRISPR/Cas9 genome editing strategy using dual guide RNAs to induce a large deletion encompassing all three uORFs. Transgenic tomato lines with the targeted uORF deletion were generated, providing a foundation for further investigation into the regulatory function of uORFs in carotenoid biosynthesis. Our findings suggest a new, transgene-free strategy for enhancing protein production through modulation of translational efficiency. Future comprehensive phenotypic and metabolic characterization of the edited lines will contribute to clarifying their potential for nutritional enhancement.

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P-10

Towards genome editing strategies in olive

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Keywords: NGTs, protoplast regeneration, *in vitro* culture

Modern biotechnological approaches have successfully been extended to a growing range of species, including woody plants, which have shown low responsiveness to *in vitro* propagation and transformation techniques compared to the herbaceous ones.

This work explores different approaches for applying New Genomics Techniques (NGTs) in olive (*Olea europaea* L.), with preliminary findings suggesting the applicability of these tools, though the development and optimization of the protocols are still necessary. The well-known recalcitrance of olive tissues to *in vitro* manipulation and regeneration of new plants represents the main obstacle to the implementation of NGTs in this species. To overcome these constraints, several methods are under investigation in our lab, including: i) the optimization of embryogenic callus culture and plant regeneration protocols specific for different genotypes; ii) the design of methods for obtaining protoplasts to be used as starting material for transfection with CRISPR/Cas9 editing agents, employing a transient delivery system based on ribonucleoproteins (RNP); iii) the plant gene editing through *de novo* meristem induction, using a Golden Gate-assembled vector with a CRISPR/Cas9 system. This multi-approach strategy will maximize the possibility of developing an efficient precision gene editing procedure in olive. Further efforts will be focused on enhancing the stability of CRISPR/Cas9 delivery systems, minimizing off-target effects, and optimizing the conditions for boosting the embryogenic competence of the cultures. As progress is made, it is anticipated that these advanced techniques will significantly accelerate the genetic improvement of olive cultivars with desirable traits useful for addressing current challenges, such as climate change-associated stresses and impact of pathogens. In particular, the developed methodologies will be applied to generate olive genotypes with increased tolerance to *Xylella fastidiosa*, a quarantine pathogen listed as one of the major phytosanitary threats throughout the Mediterranean region.

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Comparison of different gene editing methods for the introduction of targeted point mutations into corn (*Zea mays*) cell cultures

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Keywords: maize, CRISPR/Cas9, oligonucleotide-directed mutagenesis (ODM), herbicide resistance, targeted gene editing, cell cycle synchronization, chromatin modification

The development of high-performing, stress-resistant crop varieties is crucial to address the challenges posed by climate change and the growing global population. Gene editing methods represent powerful complement to traditional plant breeding techniques.

In this study, we compared two gene editing methods for the introduction of targeted point mutations into maize cell cultures: Oligonucleotide Directed Mutagenesis (ODM) and CRISPR/Cas9 system combined with template DNA.

Our target gene was the acetyl-CoA carboxylase (ACC) gene, whose targeted point mutation leads to herbicide