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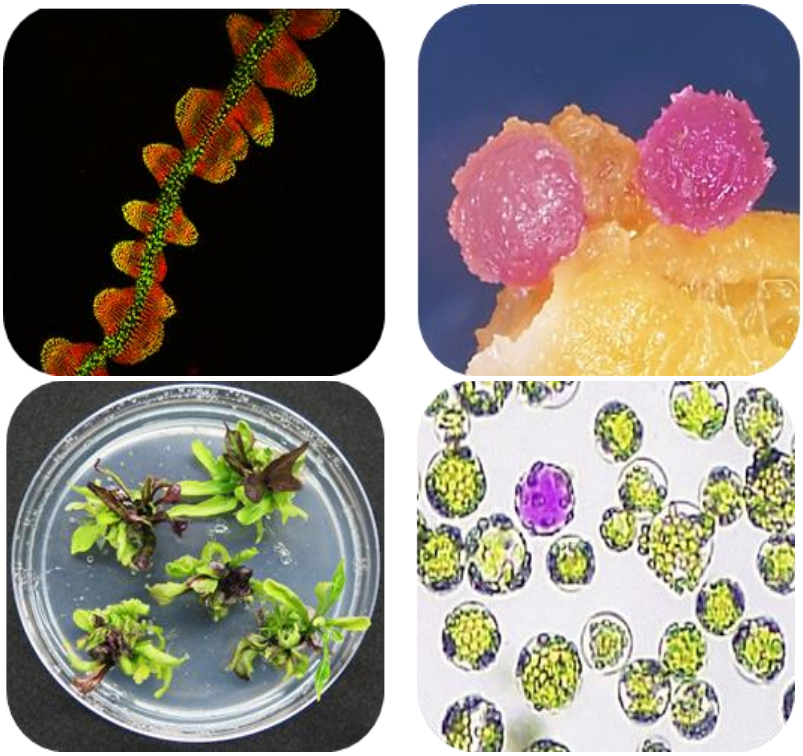
Plant Tissue Culture & Genome Engineering



Program
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ABSTRACTS
For Posters

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Unlocking the process of protoplast regeneration in Malus x domestica (apple) and phenotypic and genotypic assessment of protoplast-derived plants

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The application of advanced genetic engineering techniques to woody fruit species, frequently referred to as New Genomic Techniques (NGTs), offer the potential to provide faster and more targeted solutions for generating improved genotypes with valuable genetic traits, by overcoming some of the technical limitations of traditional breeding and the seemingly negative public perception of conventional transgenesis. Although the so-called 'DNA-free' CRISPR/Cas gene editing on protoplasts is considered as a promising technique ultimately leading to improved cultivars, mastering the regeneration process represents a bottleneck that constraints its potential to few cultivars and/or species in which it has been successfully reported. These limitations are particularly relevant for fruit tree species, such as apple, where many agronomically relevant genotypes remain recalcitrant to regeneration. The difficulty in obtaining fully regenerated plants and limited attention to potential effects derived from protoplast regeneration process have led to a scarce understanding and thorough evaluation of the phenotypic and genetic nature of resulting plants.
Our group has already proved that DNA-free editing in apple protoplasts can be achieved using CRISPR/Cas9 ribonucleoproteins, yet regeneration was not attempted. Building on our previous work, here we present a comprehensive study aimed at obtaining fully regenerated apple plants. Among key factors evaluated, we first tested the regeneration potential of a range of explant sources. Then, we chose a regenerative model to optimize liquid and semisolid culture media (including salt composition, sugar source, plant growth regulators, photoperiod), and demonstrated that fully regenerated protoplast-derived apple plants can be successfully obtained. Moreover, we phenotypically characterized protoplast-derived apple plants in glasshouse conditions, and long-read sequencing is underway to assess possible genomic effects potentially caused during protoplast regeneration. The developed workflow is currently being applied to a range of apple cultivars and other Rosaceae species, with the long-term aim of producing edited plants showing enhanced resilience-related traits.