Single Cell Technology: a step forward to New Breeding Technologies

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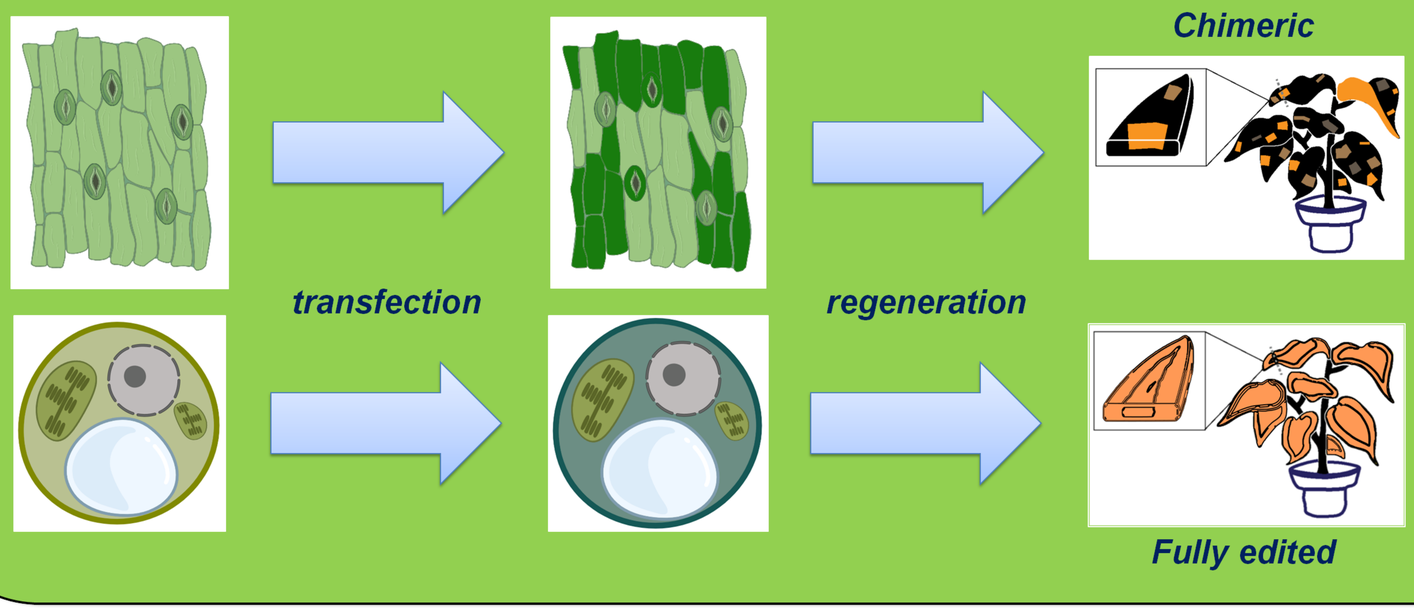
Why working with single cells?



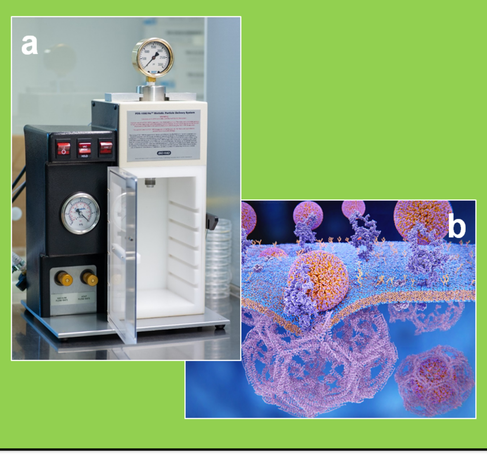
The project TRADING (*Transfer of molecular probe into plant cells for genome editing and transcriptional profiling*), funded by Fondazione Caritro, made possible the development of a new technology for the delivery of CRISPR-Cas in plant cells, by using single cells (Single Cell Technology or SCT).
Chimerism is frequently found in plants which are regenerated from tissues transfected via Agrobacterium or biolistic (top, left). Due to unhomogeneous transfection (top, center) portions of the same tissue can exhibit genetic differences which lead to chimeric plants (top, right).

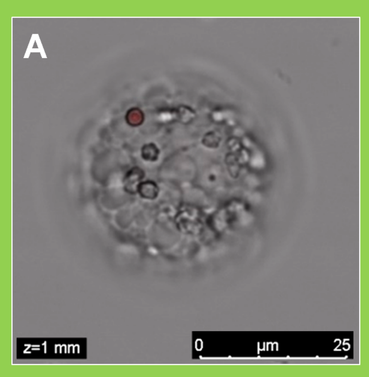
The SCT limits the extent of chimerism as a whole plant is regenerated starting from one transformed cell (bottom, left), thus securing both stability and homogeneity of its genetic makeup (bottom, center). Fully edited plants are expected as final product (bottom, right).

Adapted by Frank M.H. and Chitwood D.H., Developmental Biology, 2016, 419: 41-53.

SCT: a versatile and robust technique

Single Cell Technology was applied to different grapevine tissues. Either single, intact cells or protoplasts deprived of cell wall were obtained from embryogenic callus, as well as non-embryogenic callus and leaf tissue.
Cellular delivery of biological material (i.e. ribonucleoproteins or plasmid DNA) was carried out a) via *particle bombardment* in single cells with cell wall and b) via *liposomes* in protoplasts.

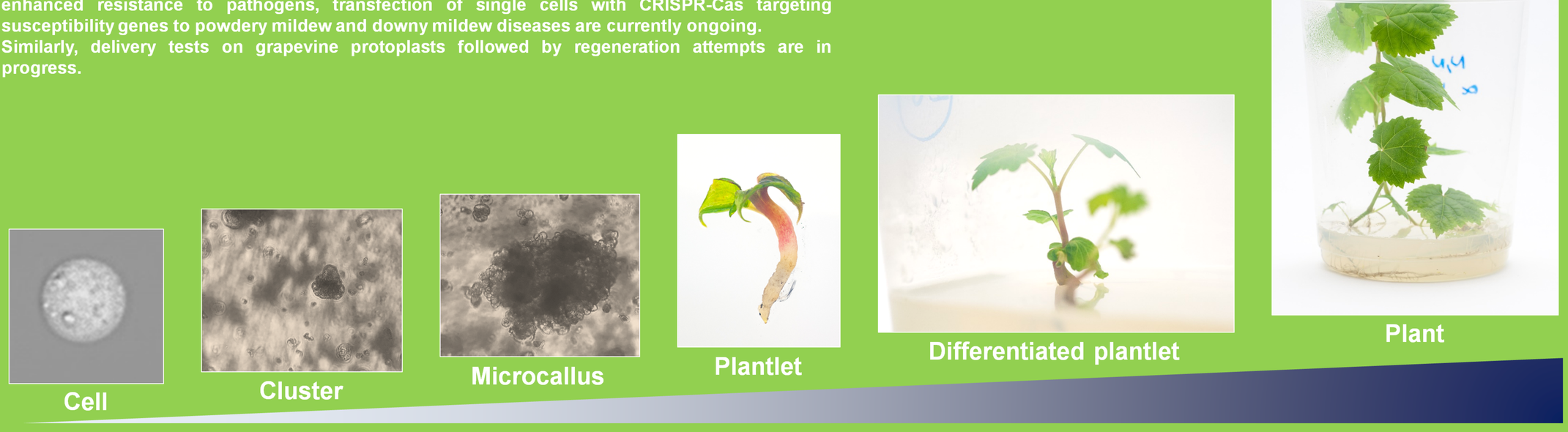




Single cells were obtained from *Vv Crimson s.* embryogenic callus. By means of particle bombardment, 2µm nanoparticles coated with the red fluorescent protein mRFP1, were delivered in single cells. Confocal fluorescence microscopy showed the red fluorescent nanoparticle localized inside a single cell (λ_{exc} 588 nm, A) and, upon FDA treatment, that the cell mantains its viability upon nanocarrier bombardment (λ_{exc} 488 nm, B). Delivery of 35S::YFP plasmid via *particle bombardment* was successful: intracellular YFP expression was detected through confocal fluorescence microscopy after 24 hours (λ_{exc} 488 nm, C).

Regenerating from Single Cells

Healthy, whole plants were successfully obtained from single cells at the end of the regenerative phase (progressive regenerative steps reported). The *Single Cell Technology* resulted promising for grapevine applications aimed at achieving new grapevine clones by exploitation of the New Breeding Technologies such as genome editing. With the aim to obtain grapevine clones with enhanced resistance to pathogens, transfection of single cells with CRISPR-Cas targeting susceptibility genes to powdery mildew and downy mildew diseases are currently ongoing.
Similarly, delivery tests on grapevine protoplasts followed by regeneration attempts are in progress.



Acknowledgements

With the contribution of

FONDAZIONE CARITRO
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Udine, 5 - 7 luglio 2021