

SINGLE CELL TECHNOLOGY: A STEP FORWARD TO NEW BREEDING TECHNOLOGIES

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CRISPR-Cas application is boosting the development of the New Breeding Technologies (NBT) aimed at obtaining precise and specific mutations in shorter times with respect to conventional breeding. The project TRADING, 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). This technology allows to limit the extent of chimerism -frequently found in plants upon regeneration from tissues transformed *via* Agrobacterium or biolistic- as a whole plant would be regenerated starting by one transformed cell, thus securing both stability and homogeneity of its genetic pool.

Both single cells with cell wall and protoplasts have been obtained by *V.v. cv* Crimson Seedless embryogenic callus. The plasmid containing the gene encoding the yellow fluorescent protein under the 35S promoter was internalized a) *via particle bombardment* in single cells with cell wall and b) *via liposomes* in protoplasts. Intracellular YFP expression was detected through confocal fluorescence microscopy. Both types of single cells were subjected to a regenerative process.

Evidences of intracellular YFP expression suggest both the successful delivery of the vector as well as its expression and the cellular viability after treatment. Furthermore, in case of single cells with cell walls the regenerative phase has been successful and healthy plants were obtained.

The *Single Cell Technology* appeared promising for grapevine applications aimed at achieving new grapevine clones by exploitation of the New Breeding Technologies such as genome editing. Tests for the delivery of CRISPR/Cas as ribonucleoprotein (RNPs), as well as regenerative tests from grapevine protoplasts, are currently ongoing.