NeosVine Project:
Green Biotechnologies to improve the Sustainability of Viticulture and Tackle Climate Change

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Introduction

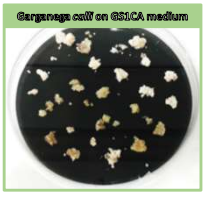
The CRISPR-Cas9 genome-editing tool and the availability of whole genome sequences offer promising solutions, enabling the introduction of targeted mutations into plants with unprecedented precision and accuracy. In breeding system, this approach allows the introduction of targeted changes (adding, removing or replacing as little as one nucleotide) to genes that control specific traits without altering organism's genetic identity. This is particularly important in crops with heterozygous genomes and commercial vegetative propagation, such as grapevine, in which traditional methods of genetic improvement that make use of sexual reproduction would disrupt the genetic and biological identity of historical varieties.

Description

NeosVine is a collaborative project between Vivai Cooperativi Rauscedo, an Italian leading grape nursery, and IGA Technology Services, a biotech company with a NGS facility.

NeosVine is characterized by the use of modern green biotechnologies, known as "New Technologies for breeding (NBPTs)" in viticulture. The NBPTs make targeted changes at the DNA level, in correspondence of genes that control specific biological properties, improving the agronomic qualities of the plants.

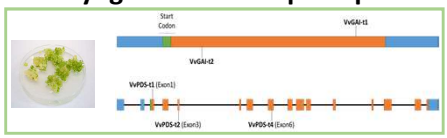
Here, we have selected three grapevine varieties for the isolation of embryogenic calli[1] and protoplasts[2]:



Garganega, Italian white-wine grape grown in the North East Italy; Syrah, is a dark-skinned grape variety; and Sangiovese, an Italian red-wine grape variety that derives its name from the Latin *sanguis Jovis*, "the blood of Jupiter".

Objectives

Adapting the CRISPR-Cas9 system to grape crop plants, we will pursue both plasmid-mediated approach and the direct delivery of DNA-free CRISPR-Cas9 ribonucleoproteins (RNPs) for inducing targeted gene silencing in embryogenic calli and protoplasts.



The validation of targeted mutagenesis and the regeneration of edited plants will be performed. We will also use 10X Genomics and Oxford Nanopore sequencing and *de novo* assembly of the edited genomes, to assess the rate of off-target mutations and any other type of somaclonal variation.

Finally, NeosVine will lay the ground for improving historical grape varieties for resistance and resilience traits and for contributing to a sustainable viticulture, supporting the wine industry with improved grape germplasm.

References

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2. Malnoy M, *et al.* **DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR/Cas9 Ribonucleoproteins**. 2016. *Front. Plant Sci.* 7:1904.

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