

Plant regeneration *via* somatic embryogenesis and preliminary trials for the application of the DNA-*free* genome editing in grapevine cv. Corvina veronese.

<u>Clarissa Ciffolillo^{1*}</u>, Edoardo Bertini², Stefania Zattoni¹, Sara Lissandrini¹, Sara Zenoni¹, Giovanni Battista Tornielli^{1,3}

¹Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy ²Edivite s.r.l. San Pietro Viminario, Quartiere San Mauro 30, 35020 Padova, Italy ³Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy.

*clarissa.ciffolillo@univr.it

Grapevine (*Vitis spp.*) is a globally significant fruit crop, and enhancing its agronomic and oenological traits is crucial to meet changing agricultural conditions and consumer demands. Conventional breeding has played a key role in domesticating grapevine varieties, but it is a time-consuming process to develop new cultivars with desirable traits for cultivation.

New plant breeding techniques (NpBTs) offer a potential revolution in grapevine cultivation, and genome editing has shown promise for targeted mutagenesis. The success of these biotechnological approaches relies on efficient *in vitro* regeneration protocols, particularly through somatic embryogenesis (SE). This method has proven successful in some *Vitis* species, but its effectiveness varies due to the genotype-dependent nature of many cultivars. Moreover, protoplasts have proven to be particularly suitable for genome editing applications, but protoplasts regeneration remains generally considered inefficient in grapevine.

The focus of this study is to enhance *in vitro* plant regeneration protocols *via* SE and isolate and regenerate plants from protoplasts derived from embryogenic calli of the Corvina veronese, a variety economically important in Veneto region. Protoplasts will serve as a platform for DNA-*free* genome editing using CRISPR/Cas9 to target genes responsible for grapevine susceptibility to powdery and downy mildew. The study includes a preliminary phenotypic characterization of regenerated plants to assess whether gene editing or the regeneration process has influenced their morphology and behaviour compared to plants grown under standard conditions. This research aims to accelerate the development of grapevine varieties with improved traits, addressing the challenges posed by conventional breeding methods.

Keywords: Vitis vinifera, Corvina veronese, Somatic embryogenesis, Protoplasts, DNA-free genome editing