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PLANT REGENERATION FROM SOMATIC EMBRYOGENESIS AND PROTOPLAST ISOLATION OF TWO VENETO REGION GRAPEVINE CULTIVARS: CORVINA VERONESE AND GARGANEGA

CIFFOLILLO C.*, BERTINI E.**, ZATTONI S.***, LISSANDINI S.****, TORNIELLI G. B.*****, POLVERARI A.******, ZENONI S.*******

*) Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

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Grapevine (*Vitis vinifera* L.) is the most economically important fruit crop worldwide, and there is considerable interest in improving its major agronomic, genetic, and enological traits in response to ever-changing agricultural environments and winegrowers demands.

Conventional breeding is an established strategy for crop improvement and has been central to the domestication of the cultivated grapevine varieties. However, crossbreeding and selection of new cultivars take many years to bring ameliorative traits to the wine industry. New plant breeding technologies (NpBT) could represent a revolutionary tool in grapevine cultivation and, in particular, genome editing through CRISPR/Cas9 system has been shown to be a valid application for targeted mutagenesis, by now on a restricted number of cultivars.

Somatic embryogenesis (SE) is a procedure where totipotent cells undergo embryogenesis pathway to form somatic embryos without fertilization. SE is an efficient model system for functional studies in many crops, including grapevine, and it represents a fundamental step for the application of NpBT. To date, this plant regeneration system is a successful tool for in vitro regeneration of some *Vitis* species but is strongly genotype-dependent, given the recalcitrance of many cultivars. Therefore, implementing *in vitro* regeneration protocols adapted to one or the other

grapevine cultivar is fundamental to applying new biotechnological approaches for genetic improvement. Protoplasts, or naked cells, are useful tool for plant biotechnology and they represent an alternative system to gene transfer methods. The protoplast isolation system is a valid approach for genome editing in many plant species, but regeneration of whole plant from protoplasts is difficult for most crops, including grapevine.

The aim of this study is to improve the *in vitro* plant regeneration protocols *via* somatic embryogenesis and the isolation and plant regeneration from embryogenic callus-derived protoplasts of two economically important Veneto region varieties, Corvina Veronese and Garganega. Subsequently, protoplasts will be then used as a platform for the application of DNA-free genome editing using CRISPR/Cas9 ribonucleoprotein complexes to perform targeted mutagenesis of genes responsible for the grapevine susceptibility to powdery mildew and downy mildew. Finally, a phenotypic characterization of the plants will be carried out to establish if gene editing and/or the regeneration process affected the morphology and the behaviour of plants obtained by this procedure compared to the plants grown in standard conditions.