Proceedings of the LXII SIGA Annual Congress
Verona, Italy – 25/28 September, 2018
ISBN **978-88-904570-8-1**

Poster Communication Abstract – 1.10

GRAPEVINE REGENERATION FROM EMBRYOGENIC-CALLI DERIVED PROTOPLASTS

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Vitis vinifera, protoplasts, recalcitrant genotypes, somatic embryogenesis, genome editing

The main bottleneck in applying cisgenesis and genome editing in grapevine is plant transformation and regeneration. Many genotypes show indeed recalcitrance to tissue culture and transformation, limiting efforts to use biotechnological approaches for grapevine genetic improvement or functional genomics studies. Grapevine embryogenic calli, induced from flower explants in tissue cultures by means of growth regulators, are used for Agrobacterium-mediated transformation, since they are tissues harbouring totipotent cells and able to regenerate transformed plants. Grapevine cultivars differ by the presence of extended structural variations that lead to extreme sequence divergence and heterozygosity, and this causes, among other things, a relatively wide variation in their potential to form embryogenic tissues. Accordingly, the availability of suitable protocols for somatic embryogenesis induction and plant regeneration in a large number of grapevine genotypes is highly desirable. In grapevine, as well as in other woody plants in which elite genotypes cannot be crossed because they would lose their unique characteristics appreciated by the consumers, the protoplast-regenerated plants represent the best starting material for genome editing purposes. In addition, the protoplast system by use of polyethylene glycol or electroporation allows the direct delivery of the genome editing machinery, such as preassembled Cas9-gRNA ribonucleoproteins, rather than plasmids encoding these components, removing the likelihood of inserting recombinant DNA in the host genome. The machinery is needed to trigger DNA repair and incorporate modifications, but it is degraded rapidly after transfection, thus reducing the frequency of off-target effects in regenerated plants.

In the present contribution, we show the setup of a protocol to regenerate *V. vinifera* cultivars starting from embryogenic calli-derived protoplasts, first step on the way to the production of DNA-free genome edited grapevines.