Proceedings of the LXVI SIGA Annual Congress
Bari, 5/8 September, 2023
ISBN: **978-88-944843-4-2**

Poster Communication Abstract – 7.27

APPLICATION OF CRISPR-CAS9 SYSTEM IN GRAPEVINE (V. VINIFERA L.) AND IMPROVEMENTS IN PLANT REGENERATION PROCESS

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Vitis vinifera L., plant regeneration, DNA-free genome editing, calli, protoplast

Currently, more and more policies are being adopted for direct agriculture towards more sustainable approaches. To continue to maintain a high production using fewer fertilizers, pesticides and water resources, agronomic techniques must be combined with biotechnological approaches.

Over the years, the socioeconomic importance of the grapevine has led winegrowers and geneticists to improve this crop to better adapt it to the environment and the market demand. However, the breeding programs are restricted by the fact that grapevine has a highly heterozygous genome, therefore, if on the one hand, we try to improve the characteristics, on the other hand it is necessary to preserve the original genome of the varieties on which the DOC heritage was founded. With the advent of NBTs it is now possible to carry out highly precise modifications leaving the genetic background unchanged. One of the newest and promising NBTs is the CRISPR-cas system.

To produce edited grapevine plants, it is necessary to dispose of both, an efficient delivery system to introduce the preassembled ribonucleoproteins (RNPs) and to ensure the regeneration of the whole modified plant. Protoplasts are the perfect platform for this purpose because they represent a system with high regenerative potential and the delivery of the RNPs can be achieved through the PEG transfection. Once the desired gene

has been edited in the single protoplast, a completely edited plant can be obtained through *in vitro* somatic embryogenesis, a process by which a single cell goes through morphological of physiological changes developing a somatic embryo. This will then continue the plant developmental program and generate shoots and roots.

In this project, we have already induced embryogenic calli to isolate protoplasts of the cultivar Glera, a white-berried variety and a crucial component of Prosecco. Protoplasts were isolated and a PEG transfection with GFP was carried out successfully indicating that the protoplasts of this cultivar are accessible to this technique. We are now proceeding with the transfection of RNP targeting susceptibility genes relative to powdery mildew and downy mildew, the most economically damaging illness of this culture. Glera, like many other varieties, is recalcitrant to the regeneration process leading to a low rate of plant recovery. In previous studies, it has been shown that the application of an external electric current enhances shoot regeneration in *N. benthamiana*. To understand whether this enhancement works also in other cultivars, here we have passed electric currents through calli generated from transgenic *A. thaliana* expressing fluorescent transcriptional reporters of transcription factors involved in shoot regeneration. Time-lapse confocal microscopy has indicated an increased expression under the current. The long-term goal is to optimise this method in grapevine and therefore face the rate-limiting step for the application of NBTs in this culture.