New breeding frontiers: application of the CRISPR-cas9 system in grapevine (V. vinifera L.) and improvements in plant regeneration

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Nowadays, many 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. In grapevine, the breeding programs are restricted by the fact that it 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. CRISPR-cas9 system is one of the smartest tools to carry out highly precise genetic modifications leaving the genetic background unchanged. To produce edited DNA-free grapevine plant is necessary to dispose of an efficient delivery system to introduce the preassembled ribonucleoproteins (RNP) and to ensure the subsequent step of regeneration of the edited plant. Protoplasts are the best system for this purpose: they represent a highly regenerative platform accessible to most of transformation techniques. The regeneration is possible through somatic embryogenesis. Grapevine is recalcitrant to the regeneration process leading to a low rate of plant recovery. The aim of this study is to optimise the *in vitro* regeneration process of *Cabernet Sauvignon* and *Glera* varieties to apply a DNA-free genome editing approach to improve agronomical and oenological traits. Here, we also provide preliminary studies on the enhancing effect of the current in the regeneration process through the application of an external electric field and evaluating the expression of fluorescent transcriptional reporters of transcription factors involved in shoot regeneration in *A.thaliana.*

Keywords: Protoplast, Somatic embryogenesis, CRISPR-cas9, *in vitro* regeneration, Electric field