P83 – Technological implementations toward successfull application of marker-free genome editing in *Vitis vinifera*

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Abstract

New Plant Breeding Techniques (*NPBT*s) protocols have been developed to produce new grape varieties with improved quantitative and qualitative characteristics. Unlike traditional cross-breeding, the NPBTs allow modifying a chosen character, keeping intact the other typical characteristics of the variety by adding or modifying only target genes.

In order to perform marker free editing, the most promising route is the one that passes by embryogenic competent callus formation, isolation of viable protoplasts, transformation with CAS ribonucleoproteins, regeneration of the plants through embryos formation.

Here, we present the recent advancement of our research team in develop genomic tools for the application of next generation molecular breeding in *Vitis vinifera* L.

The applicability of these *NPBT*s is strictly dependent to some main factors: to identify the genes involved in interesting traits, the availability of reliable transformation protocols, the possibility of avoiding unwanted integrations of exogenous DNA in the genome of the plant that would transform it into a transgenic organism, the availability of reliable regeration protocols. By combinig genomic and trascriptomics we were able to in deep study some candidate genes important in grape breeding and use these informations to precise design of gRNAs/CAS modules to target specific sites of mutation. To fine-tune the whole procedure, we chose to target *VviAGL11*, the gene that induces seedlessness in table grapes.Notewothy, we optimized methods to improve the somatic embryogenesis ability of recalcitrant genotypes, such as the Italia cultivar. Indeed, in grapes a wide application of NPBTs in *Vitis* is hindered by the null or very low aptitude to generate embryogenic calluses of many important varieties. By testing different protocols, we observed that besides the strong genotype influence, in our system the formation of total and embryogenic calluses was influenced by the type of sterilization and the cultivation substrate.

Our modified protocol increases the production of embryogenic calluses, which was a fundamental aspect for the applicability of *NPBT*s such as cisgenesis and genome editing.

We optimized a protocol for *CAS12* production and purification suitable for laboratories not specialized in the production of recombinant proteins. We also produced *CAS9* and dCAS9-VN64, a protein lacking the nuclease activity but suitable for activatation of target genes. All produced proteins were *in-vitro* tested to be fully active. Another step forward was made by improving the protocols to isolate protoplasts and to induce their transformation and regeration. We tested several protocol and strategies in order to obtain the highest possible yeld of viable protoplasts. So far, some embryo regerenants were obtained.

Keywords: Genome editing, protoplasts*, NPBT, CAS9, CAS12, VviAGL11*