Proceedings of the LXIII SIGA Annual Congress
Napoli, Italy – 10/13 September, 2019
ISBN **978-88-904570-9-8**

Poster Communication Abstract – 6.56

PLANT REGENERATION FROM PROTOPLASTS AND GENOME EDITING APPLICATION IN GRAPEVINE

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Vitis vinifera, CRISPR-Cas9, protoplast isolation, plant regeneration

Climate change affects agriculture in a number of ways, including through changes in average temperatures, rainfall, and climate extremes (e.g., heat waves). Grapevine (*Vitis vinifera* L.) is considered to be one of the major fruit crops in the world based on hectares cultivated and economic value. Grapes are used not only for wine but also for fresh fruit, dried fruit, and juice production. Recently global warming has caused anticipation of the onset of berry ripening, called veraison, with reducing grape color and increasing volatilization of aroma compounds. This change could modify the physiological characteristics of the grape, its final quality and consequently wine quality. Recently, some plant-specific transcription factors have been identified as putative regulators of the onset of ripening and preliminary results obtained from functional analysis have revealed their putative involvement in the transition from vegetative to ripening phase of berry development.

In this context, the obtainment of edited grapevine plants in which the activity of these regulators is modified in order to prevent undesirable effects of anticipating in ripening represents an important aim. The genome editing techniques have been applied in plants. In particular, the CRISPR–Cas9 genome-editing tool and the availability of whole-genome sequences from plant species have revolutionized our ability to introduce targeted mutations into important crop plants, both to explore genetic changes and to introduce new functionalities. It was recently demonstrated the possibility to apply this technique also to grapevine, however not regenerated edited plants have been obtained to date due to the recalcitrant nature of this species.

We propose an efficient protocol for whole plant regeneration from protoplasts applied in national and international varieties including Garganega, Cabernet Sauvignon, Sangiovese, Glera, Corvina, Sultana, and Pinot noir. Protoplasts will be isolated from embryogenic calli and the viability of isolated protoplasts evaluated by FDA staining and cultivated at 1*105 protoplasts/mL with the disc-culture method. Protoplast PEG-mediated transfection and YFP fluorescence analysis as well as somatic embryogenesis, transformation with Agrobacterium strain and plant regeneration will be set-up for all the selected varieties. Preliminary results show that the regeneration of the whole plant from protoplast for Glera, Sangiovese, and Sultana was done with high efficiency. Also, transformation with GFP marker in Sultana was performed successfully. The next step will be the setting-up of plasmid-mediated genome editing and the direct delivery of CRISPR–Cas9 ribonucleoproteins (RNPs) in protoplasts to achieve efficient DNA-free targeted mutations.