Proceedings of the LXVII SIGA Annual Congress
Bologna, 10/13 September, 2024
ISBN: **978-88-944843-5-9**

Oral Communication Abstract – 9.01

GENE FUNCTIONAL STUDIES AND GENE EDITING AT WORK TO IMPROVE THE SUSTAINABILITY OF VITICULTURE

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grapevine, gene editing, defense, cell wall, cuticle

Being one of the most cultivated tree crops in mid-temperate regions, grapevine (*Vitis vinifera* L.) is dangerously affected by the recent and progressive extremization of climatic conditions. This leads to an increase in favorable environments for both existing and emerging fungal pathogens. As a result, pathogen pressure on vineyards is exacerbated, which must be countered with a growing use of fungicides. Today we have the tools to enhance the resilience of this tree species and ensure sustainable production. Indeed, it is now an established practice in some laboratories to regenerate grapevine plants from gene-edited protoplasts, transfected with CRISPR/Cas9 ribonucleoprotein (RNP) complexes. The production of NGT-1 plants by DNA-free approach not only mitigates regulatory and public acceptance issues associated with genetically modified organisms (GMOs), but also ensures that the edited plants retain their original genetic background, thereby preserving varietal identity and quality. We are applying a DNA-free gene editing protocol to produce edited plants of different grapevine cultivars, while studying the function of genes whose mutations are important for the resistance to pathogens and resilience in this crop.As examples, members of the *Downy Mildew Resistance* 6 gene family were edited in two table grape cultivars, ‘Crimson seedless’ and ‘Sugraone’, to dissect their role in downy mildew (DM) resistance. Our results indicated that reduced susceptibility to DM and increased salicylic acid are obtained by knocking out both *VviDMR6-1* and *VviDMR6-2*. More recently, we obtained similar mutations in the wine cultivar ‘Chardonnay’: these prototypes are now being grafted and will be tested in the field.The editing of genes involved in the cell-wall disassembly, precisely of a member of *Pectin Methylesterase* gene family in ‘Sugraone’, proved the role

of the cell wall disassembly in the response to *Botrytis cinerea* (Bc). Increased susceptibility to *Bc* was shown upon artificial inoculation of leaves of knocked-out (KO) edited lines as compared to control plants.These results suggest that the pectin modification mediated by *VviPME10* plays an important role in the grapevine response to *Bc*. Testing the effect of *VviPME10* editing on berries and in field conditions are essential steps to determine the effectiveness of this mutation in *Bc* defense. Other traits currently under examination include transcription factors potentially regulating epidermal cell fate and cuticular wax composition in grapevine leaves and berries. These investigations pave the way for molecular breeding aimed at enhancing plant resilience, improving berry quality, and extending shelf life. Overall, testing all the plants obtained from this pipeline in field conditions is important to evaluate their performance in the light of a more sustainable viticulture.