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GENERATION OF MILDEW-RESISTANT GRAPEVINE CLONES VIA GENOME EDITING: POTENTIALS AND HURDLES

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Grapevine (*Vitis vinifera*) is one of the most important species for European agriculture, both economically and culturally. However, it is susceptible to several diseases, the most serious once being powdery mildew (PM) and downy mildew (DM) which are controlled by use of large amount of fungicides. There is urgent need to lower the impact of viticulture and the introduction of PM and DM resistant varieties can be an important step towards this aim.

The advent of New Breeding Techniques (NBTs), in particular of genome editing offers a new strategy to produce resistant plants by introducing known PM or DM resistance-genes or knocking down PM or DM susceptibility genes in commercial cultivars. In contrast to traditional breeding methods, the use of NBTs allow to modify a single gene whilst keeping the integrity of the variety intact. This is of great importance, especially for the wine industry which thrives on only a dozen of grape cultivars.

There are though some hurdles to overcome before grape cultivars created by NBTs will become practice: ao. i) identification of appropriate target genes to generate resistant cultivars, ii) develop protocols for efficient delivery of DNA or protein/RNA complexes into the cells and, iii) improve our regeneration capability to obtain plants from embryos or protoplasts. In addition to these technical problems there are regulatory uncertainties whether the products of the NBTs will be considered GMOs in Europe and other grape growing areas in the world.

Moving within this challenging framework, in the last years we have been applying genome editing to different *Vitis vinifera* commercial varieties with the aim to produce PM and DM resistant clones. To this end we knocked-out specific grapevine susceptibility genes, via gene editing technology. Embryogenic callus was transformed via *Agrobacterium tumefaciens* with CRISPR/Cas9 vectors designed to specifically edit the susceptibility genes. High efficient targeted mutagenesis has been observed in several lines regenerated from embryogenic calli, especially single nucleotide polymorphisms and/or small insertions and deletions. The edited plants have been acclimated from *vitro* to soil pots. The first DM and PM infection trials to proof validity of gene targets is in progress.