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

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Grapevine (*Vitis vinifera* L.) is one of the most important crop for European agriculture, both economically and culturally. However, it is highly susceptible to fungal diseases, such as powdery mildew (PM), causedby the ascomycete *Erysiphe necator*, and downy mildew (DM) caused by the oomycete *Plasmopara viticola*. The control of DM and PM spreading consume almost 50% of the total fungicides sprayed every year in Europe, which rises the urgent need to lower the impact of viticulture. The introduction of PM and DM resistant varieties can be an important step towards this goal.

The recent advent of New Breeding Techniques (NBTs) and in particular of genome editing offered a great opportunity to obtain resistant plants either by the introduction of known resistance-genes or by knocking down susceptibility genes in commercial cultivars. The main advantages of the genome editing approach over traditional breeding are: (1) the specificity of the mutation, which is typically limited to a single gene, helps maintaining the integrity of the parental variety and (2) the speed of the process since no several cycles of backcrosses are required.

Some hurdles need to be overcome before grape cultivars obtained by NBTs become practice: i) the identification of appropriate target genes to generate resistant cultivars, ii) the development of efficient protocols to deliver the CRISPR-Cas machinery as protein/RNA complex into single cells, and plant regeneration. In addition to these technical problems there are regulatory obstacles since for the moment in Europe NBT products are regulated under the GMO Directive.

In the last years we have tried to tackle the technical challenges by acting along two lines. We characterized susceptibility genes of the *Mlo* and *DMR6* gene families by generation of knock out mutants, in order to identify which genes are required for the establishment of the DM and PM diseases. Embryogenic callus was transformed via Agrobacterium tumefaciens with CRISPR/Cas9 vectors designed to specifically edit the candidate susceptibility genes. High efficient targeted-mutagenesis in one or two genes was observed in several lines regenerated from embryogenic calli, especially single nucleotide substitutions and/or small insertions and deletions. Edited plants grown in soil pots were challenged with DM and PM, and preliminary results highlighted a role of these genes in grapevine susceptibility to these diseases. In parallel, we also developed a new DNA-free methodology to obtain fully edited grapevine plants regenerated fromprotoplasts obtained from embryogenic callus. These plants edited in the *DMR6-2* gene, were regeneratedfrom a single edited cell, and therefore do not show chimerism,