

**PROJECT TITLE**

Quantification of the progression of powdery mildew in genome-edited grapevine plants using SignalScreen installation

**CONSORTIUM**

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## SUMMARY OF THE REPORT

The project aims to establish whether inactivation by genome editing of two susceptibility genes in *Vitis vinifera* plants is sufficient and effective in conferring resistance to *Erysiphe necator*, the causative agent of powdery mildew (PM), a devastating disease affecting grapevine worldwide. We took advantage of the EPPN 2020 opportunity to access the SignalSCREEN and SunSCREEN installations; SignalSCREEN was used to quantify the propagation of *E. necator* over time and SunSCREEN allowed to perform the infections in different environmental conditions. Together, these two installations helped to understand whether grapevine single and double mutants edited in two Mildew-Locus-O (*MLO*) genes show reduced susceptibility to the pathogen as compared to each other and to fully susceptible wild type plants of the same cultivar.

Cultivated vines are highly susceptible to *E. necator*, which causes great productive losses every year worldwide. Therefore, grape production is highly dependent on applications of fungicides, making the generation of cultivars resistant to this fungal pathogen a high priority. In several crops, *MLO* genes were associated with susceptibility to PM (Humphry et al., 2011; Wang et al., 2014). In grapevine, reduced susceptibility to *E. necator* was obtained by knock-down of multiple genes of the *MLO* family via RNA interference (RNAi) (Pessina et al., 2016). Today the genome editing era makes it possible to introduce desirable traits in crops like grapevine that are propagated via cuttings and are highly heterozygous. The genetic identity of grapevine clones is of great economical value especially in the wine-making industry, and would be otherwise disrupted by breeding. Although the technology cannot yet produce transgene-free edited plants in grapevine, in many other crops genome editing is capable of producing edited plants with no additional genetic modification other than the wanted mutation, which usually consists in one small insertion or deletion (Woo et al., 2015; Liang et al., 2019; Toda et al., 2019). In our experiment we generated transgenic lines in which the machinery for CRISPR/Cas9 editing is constitutively expressed and we were able to completely knock-out two *MLO* susceptibility genes both individually and in combination. Once properly phenotyped, these plants will serve as proof of concept for *MLO*-mediated susceptibility to PM; a key information for applications in marker-assisted breeding and New Breeding technologies such as genome editing.