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‘Clean’ genome editing in grapevine (*Vitis* spp.)Lorenza Dalla Costa^{a,*}, Loredana Moffa^a, Stefano Piazza^a, Mickael Malnoy^a^a*Via Edmund Mach 1, 38010 San Michele aAdige(Trento), Italy**** Presenting author:** lorenza.dallacosta@fmach.it

In recent years new plant breeding techniques (NPBT), and in particular genome editing via Crispr/Cas9, emerged as breakthrough tools for the genetic improvement of agricultural species, allowing to precisely modify specific genes in shorter time compared to traditional breeding and without altering the genetic heritage of cultivars. Grapevine, the most economical valuable fruit crop in the world, may receive a major benefit from NPBT since viticulture is based on a few elite varieties. However, to date the European Commission (EC) has not yet deliberated on the legal status of the NPBT products, whether they should or should not be covered by GMO legislation (Directive 2001/18). Waiting for the EC decision, we applied the Crispr/Cas9 system in grapevine for the inactivation of the *VvMLO7* gene which plays a key role in susceptibility to powdery mildew. Our “clean” strategy aims at leaving in the plant genome the minimal trace of exogenous DNA. It uses the classical *Agrobacterium tumefaciens* (A.t.) to introgress Cas9, the sgRNA and the selection marker gene nptII and allows removing the T-DNA cassette from the grapevine genome once the targeted mutations have been obtained. To this purpose, the FLP recombinase gene under the control of a heat-shock inducible promoter has been integrated in the T-DNA as well as its recognition sites (FRT), placed next to the A.t. left and right borders. NptII- and Cas9-positive lines of ‘Chardonnay’, ‘Thompson Seedless’ and ‘Microvine’ were analyzed by next generation sequencing in order to assess the induced mutations in the target site. Subsequently, the site-specific removal of the T-DNA cassette was evaluated in the heat-treated lines by quantifying nptII copy number with a Real-time PCR method. The effect of powdery mildew infection on *VvMLO7*-edited plants is currently under evaluation.

Keywords: FLP/FRT system, genome editing, marker-free, powdery mildew, *VvMLO*