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# EFFICIENT AND 'CLEAN' CRISPR/CAS9 EDITING FOR FIRE BLIGHT RESISTANCE IN APPLE

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Fire blight, caused by the bacterium *Erwinia amylovora* (*E.a.*), is one of the most economically important and invasive diseases affecting apple (*Malus x domestica*). Nowadays, the *E.a.*-apple interaction is widely described and the main molecular mechanisms underlying both plant host resistance and *E.a.* pathogenesis are elucidated. Nevertheless, the management of the disease remains still ardous in apple orchard. Indeed, the use of genetically modified plants is forbidden by the current GMO legislation and the application of antibiotic-based pesticides is prohibited due to their effects on human health and environment. Therefore, one of the hardest challenges for plant biotechnology is to provide apple varieties that are simultaneously disease-resistant and public-acceptable. In this study, conducted on two *Malus x domestica* susceptible varieties, 'Royal Gala' and 'Golden Delicious', a genome editing approach based on CRISPR/Cas9 via *Agrobacterium tumefaciens* (*A.t.*) was applied to produce the knock-out of *MdDIPM4*. In apple, evidences show that MdDIPM4 protein interacts specifically with the DspE effector, mandatory for the pathogenesis of *Erwinia amylovora*. About sixty transgenic lines were analyzed using an high throughput screening approach on the Illumina MiSeq platform in order to verify the CRISPR/Cas9-induced mutations. The eighty percent of the plants showed mutations, especially translation-terminated deletions resulting in *MdDIPM4* knock-out. Moreover, with the aim of producing public-acceptale engineered apple plants, our strategy allowed to remove the entire exogenous T-DNA in those lines selected for the desired mutation. This mechanism relies on the heat schock-inducible expression of the *Flp* gene which induces a site-specific recombination at the two FRT sites flanking the *A.t.* left and right borders. Some genome edited lines were heat-treated and the removal of a 10.5 kb T-DNA cassette was proved. Currently, the selected genome edited and T-DNA-free apple plants are under *in vivo* investigation to test their resistance to *Erwinia amylovora*.