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IMPLEMENTING THE CRISPR/CAS9 SYSTEM FOR TARGETING SHARKA RESISTANCE IN PEACH

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In perennial crop species, the release of a new variety is a lengthy and costly process, which may take up to 30 years. With the advent of the genomic era, advancements in the identification and isolation of causal genes have paved the way to novel approaches, collectively referred to as New Breeding Techniques (NBT), which allow to dramatically reduce the time of genetic improvement.

Functional genomic resources have been developed for the *Prunus* genus together with stable or transient genetic transformation protocols. The availability of all these tools enables the application of the genome editing approach via the CRISPR/Cas9 system to develop stone fruit plants resistant to sharka. Candidates to target are susceptibility genes coding for factors that are required for the pathogen infectious cycle while also having a constitutive role in the cell and/or plant life. Recently, many different plant factors required for the Plum pox virus infection cycle have been identified.

Highly regenerative systems for explants production and whole plant regeneration are key steps of fruit tree genetic transformation. In most woody fruit species, transformation and adventitious regeneration are difficult, with low efficiency and often limited to a few genotypes or to seed derived tissues. This is particularly true in the *Prunus* genus.

In the present work, in the frame of a Marie Curie Skłodowska RISE H2020 action (RISE-TESS), CRISPR/Cas9 cassettes targeting Sharka susceptibility genes developed by INIA La Platina station in Santiago (Chile) and the pLSLGFP.R (Addgene #51501, Baltes et al. 2014) plasmid, containing the reporter *gfp* gene, were used to transform peach tissues by *Agrobacterium tumefaciens* infection. Moreover, the CRISPR/Cas9 cassette, was carried by two different vectors: a T-DNA based vector and a geminivirus-derived one. Cotyledons and epicotyls from seeds of cvs Rich Lady, Royal Glory and Royal Majestic were used. Regeneration events from zygotic-derivative tissues were obtained after infection and are under evaluation for actual transformation. Alternative regeneration strategies in *Prunus*, independent from seasonality and starting from clonally derived material, using micro-propagated *Prunus* cultivars, are also being approached.

The work, funded by the Italian Ministry of Agriculture under the Biotech project, is in progress to molecularly assess explants transformation and putative editing events in the target

genes compared to the wild type. Besides, development and improvement of efficient *in vitro* protocols is still ongoing.