

CRISPR/Cas9-mediated editing of the *TM6* MADS-box gene in the octoploid strawberry (*Fragaria x ananassa*)

Carmen Martín-Pizarro¹, David Posé¹

1) Laboratorio de Bioquímica y Biotecnología Vegetal, Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM), Universidad de Málaga-Consejo Superior de Investigaciones Científicas. Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias.

The B-class of MADS-box transcription factors has been studied in many plant species, but remain functionally uncharacterized in the Rosaceae family. APETALA3 (AP3), a member of this class, controls the identity of petals and stamens in *Arabidopsis thaliana*. In this work, we identified two members of the AP3 lineage in the cultivated strawberry (*Fragaria x ananassa*): FaAP3 and FaTM6. Interestingly, FaTM6, and not FaAP3, shows an expression pattern equivalent to that of AP3 in *Arabidopsis*. Genome editing using Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system is becoming a robust tool for targeted and stable mutagenesis of DNA. However, whether it can be efficiently used in an octoploid species such as *F. x ananassa* is not known. In our study, we report the application of the CRISPR/Cas9 in *F. x ananassa* to characterize the function of FaTM6 in flower development. An exhaustive analysis by high-throughput sequencing of the FaTM6 locus spanning the target sites showed a high efficiency genome editing already in the T0 generation. The phenotypic characterization of the mutant lines indicates that FaTM6 plays a key role in petal and especially in anther development in strawberry. Our results validate the CRISPR/Cas9 strategy for gene functional analysis in an octoploid species such as *F. x ananassa*, and offer new opportunities for engineering strawberry to improve traits of interest in breeding programs.