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

Carmen-Martín Pizarro and David Posé

Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga–Consejo Superior de Investigaciones Científicas, Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain.

The B-class of MADS-box transcription factors has been studied in many plant species, but remain uncharacterized in Rosaceae. APETALA3 (AP3) is a member of this family, controlling petal and stamen identities in Arabidopsis. In this study, we have identified two members of the AP3 lineage in Fragaria x ananassa: FaAP3 and FaTM6. In this work, we used the CRISPR/Cas9 system for the first time in an octoploid species in order to study the function of TM6 in strawberry flower development. At the same, this study allowed us to validate the efficiency of the CRISPR/Cas9 system in an octoploid species. Analysis by high-throughput sequencing of FaTM6 locus in the tm6 mutant lines showed high efficiency of the edition in T0 generation. Phenotypic characterization of the mutant lines revealed that FaTM6 plays an important role in petals, but especially in anther development, which was drastically impaired. This defect was accompanied by a reduction in pollen content and viability, resulting in fruit abortion in the tm6 mutant lines. In conclusion, our study not only has shed light on the biological function of FaTM6 in flower development but also has shown the potential of CRISPR/Cas9 for improving traits of agronomic interest despite the high ploidy of the cultivated strawberry.