Genome editing of the octoploid *Fragaria* × *ananassa* using the CRISPR/Cas9 system

Carmen MARTÍN-PIZARRO[(1)], David POSÉ[(1)]

[1] Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga-Consejo Superior de Investigaciones Científicas, Ed. I+D, 3ª planta, Campus Teatinos s/n, 29010, Málaga, Spain.

Email de contacto:dpose@uma.es

Due to its octoploid nature, gene functional analyses in the cultivated strawberry (*Fragaria* × *ananassa*) are commonly carried out via gene silencing using self-complementary "hairpin" double-stranded RNA (RNAi) constructs. However, this system is not always as efficient as expected. First, an efficient silencing of the target gene is not always achieved, and second, its effect might not be stable after several clonal propagations of the transgenic lines. Recently, genome editing is becoming an important biotechnological tool for gene functional analysis and crop improvement, in particular since the development of the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat-CRISPR associated protein 9) system.

To investigate the functionality of the CRISPR/Cas9 in strawberry, we designed two sgRNAs directed against two regions of the floral homeotic gene *APETALA3* (*AP3*) in order to induce a deletion of around 200 nt. A vector containing both sgRNAs and Cas9 was used to transform leaf disks of *F.* × *ananassa* cv. Camarosa. Several independent stable transgenic lines displayed defects in stamen and fruit development, partially phenocopying that of the Arabidopsis *ap3* mutants. Molecular analysis of the targeted *AP3* locus indicated differences in gene editing among different transgenic lines and suggests mutations in all the possible *AP3* alleles. Phenotypic analyses indicate that impaired fruit development might be caused by the lack of proper development of the anthers due to the CRISPR/Cas9 induced mutation in *AP3*.

In summary, we show that the CRISPR/Cas9 system is a functional tool to perform genome editing in the octoploid *F.* × *ananassa*. We propose this system as an alternative to the traditional RNAi strategy to stably mutagenize a particular gene of interest for functional analyses in this species.