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Targeted Mutagenesis of *FaTM6* in the Octoploid Strawberry (*Fragaria x ananassa*) using the CRISPR/Cas9 system

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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 × 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. × ananassa* is not known. In our study, we report the application of the CRISPR/Cas9 in *F. × 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. × ananassa*, and offer new

opportunities for engineering strawberry to improve traits of interest in breeding programs.

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