

Functional Analysis of TM6 MADS-Box Gene in the Octoploid Strawberry By CRISPR/Cas9 Directed Mutagenesis

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The B-class of MADS-box transcription factors has been studied in many plant species, but remain functionally uncharacterized in Rosaceae. APETALA3 (AP3), a member of this class, controls petal and stamen identities in *Arabidopsis*. In this study, 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 CRISPR/Cas9 system is becoming a robust tool for targeted and stable DNA mutagenesis. However, whether it can be efficiently used in an octoploid species such as *F. × ananassa* is not yet established. Here we report the application of CRISPR/Cas9 to characterize the function of FaTM6 in strawberry flower development. An analysis by high-throughput sequencing of the FaTM6 locus spanning the target sites showed a highly efficient genome editing already in the T0 generation. The phenotypic characterization of the mutant lines indicates that FaTM6 plays a key role in anther development in strawberry. Our results validate the CRISPR/Cas9 strategy for gene functional analysis in *F. × ananassa* as an octoploid species, and they offer new opportunities for engineering strawberry to improve traits of interest in breeding programs.