

Genome Editing of the Octoploid *Fragaria* × *Ananassa* Using the CRISPR/Cas9 System

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Gene functional analyses in the cultivated strawberry (*Fragaria* × *ananassa*) are commonly carried out via gene silencing using intron hairpin RNA (ihpRNA)-based constructs. However, this system is not always as efficient or stable as expected. As an alternative, we investigated the functionality of the CRISPR/Cas9 system in this octoploid species, targeting the floral homeotic gene APETALA3 (AP3). Several independent lines displayed defects in stamen and fruit development, partially phenocopying 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 eight AP3 alleles for some of them. Importantly, these mutations were maintained in clone plants generated from runners, ensuring the maintenance of the CRISPR/Cas9 editing during strawberry plant propagation.

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