

## Efficient dual-gene targeting *AGAMOUS*-like genes in domestic apple

Targeted genome editing has made it possible to obtain precise changes in one or more genes of interest in a variety of plant species. Application of this technique will benefit from increased knowledge of the efficacy of the approach and the potential impact of a retained CRISPR-Cas9 editing system in species where breeding away the transgene is not practical. One example is domestic apple, a valuable fruit crop with an extended juvenile period and obligatory outcrossing. Here, we used CRISPR-Cas9 constructs with two guide RNAs to target two *AGAMOUS*-like genes in two cultivars of domestic apple. Of the 38 CRISPR-Cas9 transformed events obtained, most events (35, 92%) had at least one genetic change, and 13 events (37%) had changes to all 4 target alleles. Characterization of mutation types revealed that most changes were large or small deletions. We did not observe any inversions or large insertions. Many of the mutations obtained were predicted to alter the reading frame and lead to early stop codons. As trees varied in both number and type of altered alleles, the full phenotypic impact of the genetic changes will likely cover a spectrum of phenotypes. As both targeted genes are predicted to be key for floral organ identity and floral meristem determinacy it is anticipated that the flowers will vary in form and morphology. The trees are currently in their juvenile phase, it will be some time before floral features can be observed. As the trees have a retained CRISPR-Cas9 editing system, this additional time will provide an opportunity for determining if there is continued CRISPR-Cas9 activity in trees with currently un-edited alleles.

### Keywords

CRISPR-Cas9; domestic apple, *AGAMOUS*; genome editing; mutation