Title: 3022- Implementing the CRISPR/Cas9 editing in kiwifruit: a first step towards a hermaphroditetetraploid valuable cultivar

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Abstract body text:
Actinidia species are apparently all functionally dioecious. Dioecism creates inevitable disadvantages in breeding, such as the inability of determining the breeding values for fruit characters of paternal parents. Cross-populations produce female and male offspring in a 1:1 ratio, with male plants representing a waste of land and resources since the selection focuses, usually, on fruit-bearing individuals. Gender inconstancy has been described in some genotypes. However, breeding cannot be based on such genotypes being such incipient hermaphroditism erratic. Developing stable hermaphrodite cultivars would offer great advantages, whilst overcoming the inevitable problems that dioecism poses to the crop management. In the present study, we induced a targeted mutagenesis of SyGI gene (one of the two putative sex determinants) exploiting a CRISPR/Cas9 multiplexing vector and two different paired-guide RNAs localized in two different sites of the SyGI coding sequence, in order to induce a stable knock-out in two tetraploid male accessions of Actinidia chinensis var. chinensis (A0134.41 and Ac174.46). Tetraploid fruiting genotypes usually have better stress resistance, bigger fruit and faster growth speed compared to diploids. Therefore, stable hermaphrodite tetraploid genotypes would be a good achievement for kiwifruit breeder. Despite we are not yet able to verify the phenotypic effects of the editing on the flower structure, due to the long time required by tissue-cultured kiwifruit plants to flower, the screening of edited mutations resulted in several regenerated lines showing a near fixation of a unique modification in their genomes. The onset of a premature stop codon, which induces the putative gene knock-out, was observed. Sequence analysis of the target locus of one guide also highlighted a co-amplification of a minor nucleotide variant differing from the target region for a single nucleotide, which could lead to the hypothesis of a genomic duplication in proximity of the Y genomic region.