Overcome dioecism in tetraploid kiwifruit: a CRISPR/CAS9 editing approach

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Kiwifruit belongs to the genus *Actinidia* with 54 species apparently all functionally dioecious. The sex-determinants of the type XX/XY with male heterogametic, operate in all taxa and independently on the ploidy level. Dioecism is not absolute, and male vines bearing small fruit have been described. However, breeding cannot be based on such genotypes being such incipient hermaphroditism erratic. The possibility to develop stable hermaphrodite cultivars would offer great advantages, whilst overcoming the inevitable problems that dioecism brings. Recently, the SyGI protein has been described as a Y-encoded cytokinin response regulator that acts as one of the two putative sex determinants, being the suppressor of female development. In the present study, we exploited a CRISPR/Cas9 multiplexing vector, and paired-guide RNAs (gRNA1 and gRNA2) targeting two different site of SyGI gene in order to induce a stable gene knock – out in two tetraploid male accessions of *Actinidia chiensis* var. *chinensis* (A0134.41 and Ac174.46). The two genotypes showed a regenerative efficiency of 58% and 73% respectively. Despite not yet being able to verify the phenotypic effects on the flower structure, due to the long time required by tissue-cultured kiwifruit plants to flower, the evaluation of editing mutations highlights two regenerated lines (A0134.41_L3 and Ac174.46_L1) showing near fixation of a unique modification in their genomes, resulting in both cases in the onset of a premature stop codon which induces the putative gene knock out. Editing evaluation of gRNA1 locus for both regenerated lines resulted in co-amplification of a minor nucleotide variant differing from the target region for a single nucleotide. A genomic duplication of the region in proximity of the Y genomic region could be postulated.

Keywords: *Actinidia* spp., plant transformation, sex-determinant, new breeding technologies (NBTs).