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HERMAPHRODITISM IN KIWIFRUIT: LOSS OF SHYGI ACTIVITY INDUCED BY CRISPR/CAS9 EDITING

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Kiwifruit, a major fruit crop worldwide, belongs to the genus Actinidia and most species are characterized by functional dioecism. This means that male individuals bear fertile anthers and rudimental carpels, and female individuals bear fertile carpels and regular but functionally-sterile anthers. Due to its dioecism, kiwifruit has an inevitable disadvantage in breeding; paternal parents are selected with unknown fruit quality since male plants cannot bearing fruit. In a typical cross population, dioecism results in a final sex ratio of 1:1 for female to male vines. Therefore, these male plants can represent a part of the progeny that are a waste of land and resources. Dioecism is not absolute, and male vines bearing small fruit have been described, but being such incipient hermaphroditism erratic, breeding cannot be based on such genotypes. Recently, Akagi and co-workers (2018) described the ShyGI protein, which is a Y-encoded cytokinin response regulator and acts as one of the two putative sex determinants, the suppressor of female development.

In the present study, we exploited the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system to target the ShyGI gene, in order to induce stable hermaphroditism in a male accession of Actinidia chiensis var. chinensis. We used the pDIRECT_22C (Cermak et al., 2017), a CRISPR/Cas9 multiplexing vector, and two guide RNAs (gRNA1 and gRNA2), designed using the software Cas-Designer (Park et al. 2015). The two gRNAs were selected to target the first and second exon of the gene, and they were cloned into pDIRECT_22C vector within an array controlled by an RNA Pol II promoter, using an approach based on Golden Gate assembly. The assembled vector was cloned into A. tumefaciens LBA4404 strain and used to agro-infiltrate plant leaves of the Actinidia male accession, in order to perform a preliminary genome-wide sequence analysis of specific and off-target editing effects of the two gRNAs. Eventually, the A. tumefaciens strain carrying the assembled vector will be used to co-cultivate leaf discs of the same plant material, in order to obtain stable transformants.