CRISPR-Cas9-Based Modification of Tomato Metabolism for Improved Varieties

Tomato (Solanum lycopersicum) is an agriculturally significant crop, with an annual production of 200 million tons globally and a 1.3-1.67 billion industry in the US. Tennessee ranks seventh in the U.S. for acres planted and harvested, the state comes in third nationally for production value with $54 million, behind California and Florida in 2018.

Modern tomato varieties include small-fruited types (such as cherry tomatoes), large-fruited types (like Beefsteak), and Roma tomatoes, which produce fruit sizes in between. This research focuses on modifying the genomes of tomato varieties using CRISPR-Cas9 gene-editing technology to create new lines with improved fruit properties. The AP2a (APETALA2a) gene is an ethylene-responsive transcription factor that is highly expressed in tomato fruits at the breaker stage, when fruits begin ripening and softening. This gene plays a key role in accelerating the fruit ripening process.

We prepared the gene-knock-out CRISPR/Cas9 construct with three gRNAs and transformed into *Agrobacterium tumefaciens* GV505 strain. Three tomato varieties, including 'Money Maker' (round tomato), 'Micro-Tom', and LA2876 (cherry tomato) were used to generate the gene-edited lines. Tomato seeds were sterilized with 30% bleach, germinated on ½-strength MS medium. Genetic transformation was conducted using cotyledons from 6–8-day-old seedlings which were inoculated with *Agrobacterium tumefaciens* harboring the CRISPR/Cas9 - AP2a KO construct. Shoots regenerated on selection media (hygromycin) were sub-cultured monthly. Leaf tissues were collected and genomic DNA was extracted using the CTAB method. Transgenic plants were confirmed by PCR using primers covering the Cas9-gRNA region. Gene-edited events were confirmed by PCR using the AP2 gene specific primers. Our results showed that all of 21 regenerated lines contained the Cas9-gRNA region and they are transgenic plants. Then Sanger sequencing analysis of the PCR products amplified using the AP2 gene specific primers confirmed that 17 individual lines have mutation in the targeted regions. The transgenic to gene-edited ratio is 21:17. The edited gene would produce truncated proteins which will not translate into the functional protein. These knockout tomato lines will be evaluated for ripening and softening properties. The work was conducted in collaboration with a private company (Lifeasible, NY). This project was supported by USDA NIFA grant Proposal Number: 2021-12803 and USDA NIFA 2022-38821-37353.