Generation and characterization of CRISPR-Cas9 gene edited tomato lines

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. Tomato yield and quality are severely affected by suboptimal environmental conditions. In plants, basic helix–loop–helix (bHLH) transcription factors have a role in responses to a wide array of abiotic and biotic stress factors. The entire tomato genome contains a total of 152 bHLH transcription factors. Our studies have identified several candidate genes affecting responses to saline and acidic soil conditions and heat stress. This project aims to develop gene-edited lines targeting these candidate genes using CRISPR-Cas9 system and select for stress tolerant genotypes. Custom-designed guide RNAs (gRNAs) were synthesized and integrated into the pDIRECT_23C binary vector and then transferred into Agrobacterium strain AGL1. Tomato explants were inoculated with Agrobacteria carrying the plasmids, and shoots regenerated on selective culture plates were identified as putative transgenic plants. The integration of T-DNA regions in the tomato genome was verified using PCR analysis of the Cas9 and gRNAs regions. The sequences of the targeted genes were amplified and sequenced using Sanger sequencing. Six individual transgenic lines were confirmed to have insertion/deletion in the gRNA1 targeted region of a bHLH transcription factor (Solyc01g058670.2). These putative gene edited plants (T0) were rooted, transplanted into greenhouse, they were verified for gRNA inserts in the genome and stability of gene mutation. Non-transgenic mutant plants are selected in T1 and T2 generations followed by phenotyping for stress tolerance. Future research will characterize these gene edited lines for tolerance to salt stress, soil alkalinity, and heat stress.