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Developing gene editing system for tomatoes to generate stress tolerant lines

Tomato (Solanum lycopersicum) is an important vegetable crop worldwide, it is also an model plant for genetic/genomic researches. The clustered regularly interspaced short palindromic repeat/CRISPR-associated protein (CRISPR/Cas)-mediated genome editing technology enables efficient targeted modification and has great potential for breeding desired plants with resistance to abiotic and biotic stresses. In this study, four genes (ALTF, PUR, BHLH, RAF) related to tomato tolerance to abiotic stresses including acidic pH and Al toxicity, heat stress and soil saline conditions, were selected. These gene sequences were retried from the tomato genome database. The localized expression of these genes in plants were determined using the community resource website for gene expression. None of the selected genes are highly expressed in leaf tissues, the knock-out of these genes should not have strong ectopic effects affecting normal plant growth. The gRNAs were designed, synthesized and cloned into the pDIRECT_23C binary vector. After validation by sanger sequencing, the plasmid was transferred into Agrobacteria tumefaciens strain AGL1. Positive clones were confirmed using PCR and plasmid sequencing analysis. Tomato cotyledons were inoculated with the Agrobacteria harboring the respective plasmid. Shoots regenerated on selection plates containing Basta are considered as putative transgenic plants. Insertion of the T-DNA containing Cas9, gRNA into tomato genome were validated using PCR and sequencing. Positive plants are rooted and transplanted into greenhouse to select non-transgenic, gene-edited tomato plants.