

## ENGINEERING WATER USE IN TOMATO BY GENERATING NOVEL ALLELIC VARIATION OF ABA SIGNALING GENES VIA CRISPR-CAS9

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The enormous demand for water by world agriculture increases rapidly. There is therefore an urgency to generate crops with more efficient water use. Tomato is a major crop plant, and it is becoming very important to identify genotypes with superior productivity a fruit quality with reduced use of resources. CRISPR-Cas9- based approaches provide us with the tools for targeted mutagenesis approaches (knockout and gene editing) of genes that modulate pathways related to abiotic stress responses. Absciscic acid (ABA) is the key signal molecule that mediates the acclimation of plants to water deficit by stimulating the closure of stomata, thus reducing transpiration (Munemasa et al., 2015) and by activating various metabolic and physiological adjustments that allow maintenance of cellular turgor (Finkelstein, 2013). Increasing ABA sensitivity may therefore represent an obvious strategy for improving water use efficiency (WUE, the biomass produced per unit of water) in cultivated plants, although a constitutively exaggerated ABA response may penalize plant growth and productivity. The goal of our research is to generate a wide allelic variability at different components of ABA signaling to improve WUE and minimize the growth penalty. This work is in the frame of a larger project (Cisgenesis and genome editing in tomato – CISGET) that includes several partners harnessing the great potential of new breeding technologies to accelerate genetic improvement programs in tomatoes.

The target genes modified via gene editing are (i) the putative ortholog of the tomato AtMYB60 gene, which functions as a positive regulator of

stomatal opening, and (ii) a family of genes encoding type 2C phosphatase proteins (PP2C), which are negative regulators of ABA signaling. Phylogenetic and functional analyses demonstrated conservation of the MYB60 protein in various cultivated species, including tomato (gene *SlMYB60*) (Rusconi et al., 2013). The PP2C gene family of tomatoes is highly expanded (92 genes) (Qiu, J. et al. Genes 2022). However, we chose to focus on PP2Cs that are transcriptionally activated by water deficit, and with high levels of evolutionary conservation with the best-characterized PP2Cs of *Arabidopsis* (e.g. *ABI1/2*, *HAI1*). On this basis, the genes *SlPP2C1*, *SlPP2C2*, *SlPP2C4*, and *SlPP2C5* have been edited in the same plant, while other lines have been selected for *SlMYB60* gene editing events. After *Agrobacterium*-mediated transformation, we recovered 10 independent T0 plants for both sets of targets across 3 different backgrounds (Ailsa Craig, M82, and Red Setter). Sequencing of T0 Cas9-positive plants revealed that all the target genes contain at least one mutated allele, frequently in a homozygous bi-allelic state. This provides us with a large pool of alleles that can be mined for water use-related traits and to identify specific combinations that confer a tradeoff between water deficit tolerance and growth performance.