CRISPR/CAS9 EDITING OF PROLINE METABOLISM AND SOS PATHWAY GENES FOR IMPROVING ABIOTIC STRESS TOLERANCE IN TOMATO

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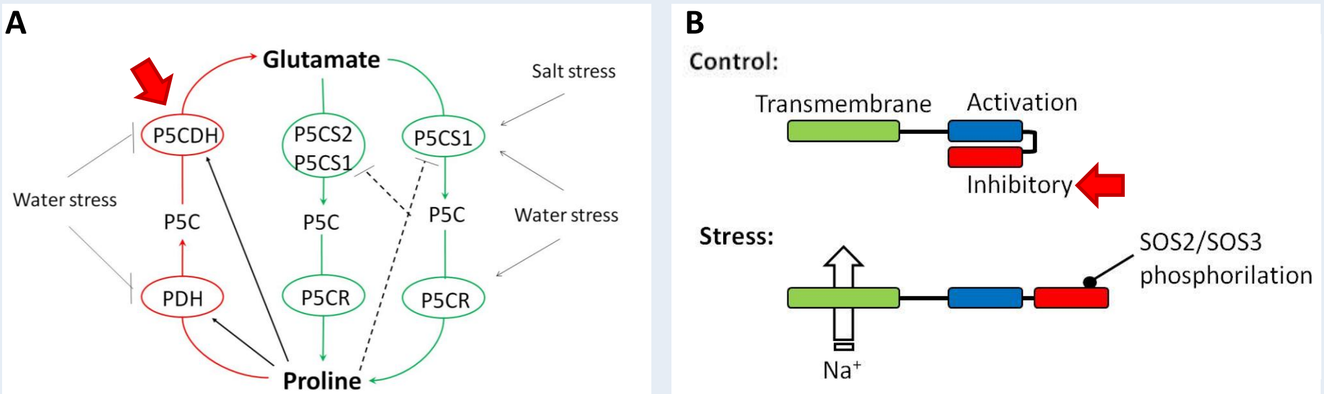
Rationale

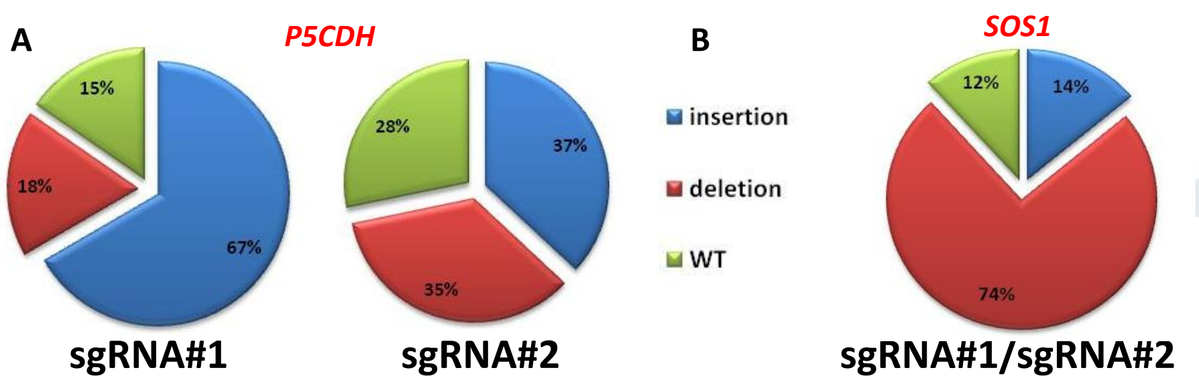
Drought and salinity stresses cause plant growth reduction and crop yield loss. Here, we edited genes involved in proline metabolism and Salt Overly Sensitive (SOS) pathway to obtain stress-tolerant tomato (*Solanum lycopersicum L.)* plants.



Selected targets (**A**) Proline metabolism in plants. Proline biosynthesis (green lines) and degradation (red lines) are reported. (**B**) Schematic representation of the SOS1 protein and its activation mechanism. Red arrows indicate the targets: the *P5CDH1* (Solyc06g071000) gene (**A**) and the inhibitory domain of *SOS1* (Solyc01g005020) (**B**).

Evaluation of sgRNA efficiency

We designed two sgRNA guides for each target. These were located in the first and third exon of *P5CDH1*, and in the exon encoding the *SOS1* inhibitory domain. The efficiency of editing was evaluated through the hairy roots system. Genotyping of roots by high resolution fragment analysis (HRFA) showed high mutation efficiency at target sites.

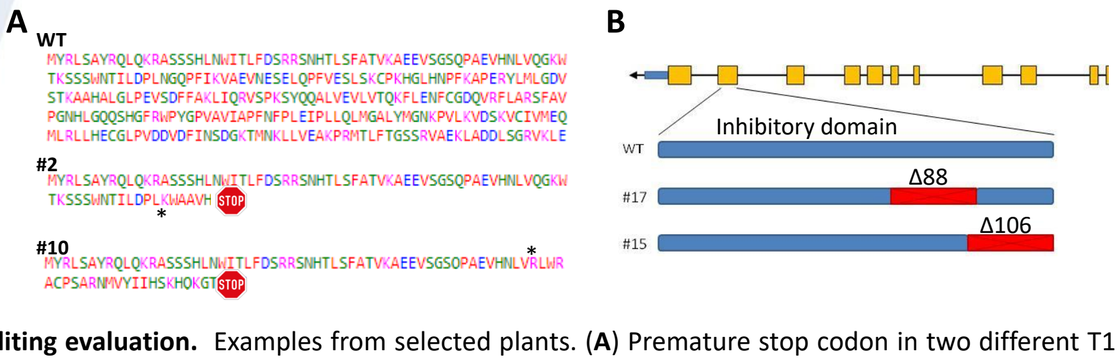


Evaluation of sgRNAs efficiency. HRFA results: percentage of deletions and insertions at each target site *P5CDH1* (**A**) and *SOS1* (**B**) was reported.

Stable transformation

Genotyping

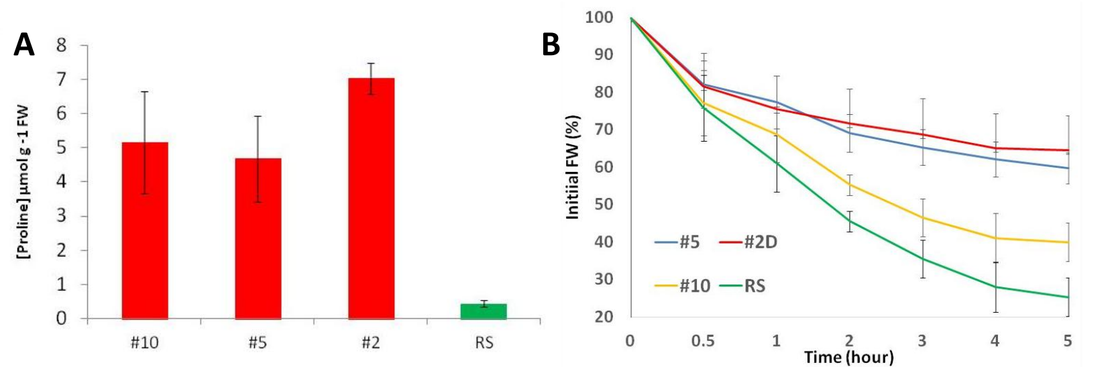
Agrobacterium tumefaciens-mediated transformation of *S. lycopersicum* cotyledons (cv. Red Setter) was performed to obtain stable transformed plants. Through PCR and DNA sequencing, we selected mutations resulting in premature stop codons in *p5cdh1* and large deletions in the inhibitory domain of *sos1.*



Editing evaluation. Examples from selected plants. (**A**) Premature stop codon in two different T1 *p5cdh1 (*#2, #10) lines (**B**) Deletion (Δ, red box) in the inhibitory domain in two different T1 *sos1* (#17, #15) lines . WT: wild type .

Phenotyping

Preliminary phenotyping of progenies of independent *P5CDH1* transformants showed a larger amount of proline in leaves and decrease in the transpirational water loss compared to wild type.



Proline quantification and water loss. Leaf proline content (**A**) and water loss of detached leaves (**B**) of progeny of three different T1 *p5cdh1 (*#10, #5, #2) lines and wild type (RS: Red Setter).

Conclusions

These results indicate that the tested sgRNAs can successfully edit the *P5CDH1* and *SOS1* tomato genes. Phenotyping of *p5cdh* and *sos1* edited plants in stress conditions is in progress to verify the improved stress tolerance.