CRISPR-based assessment of *SlGRAS10* function during tomato fruit development and ripening

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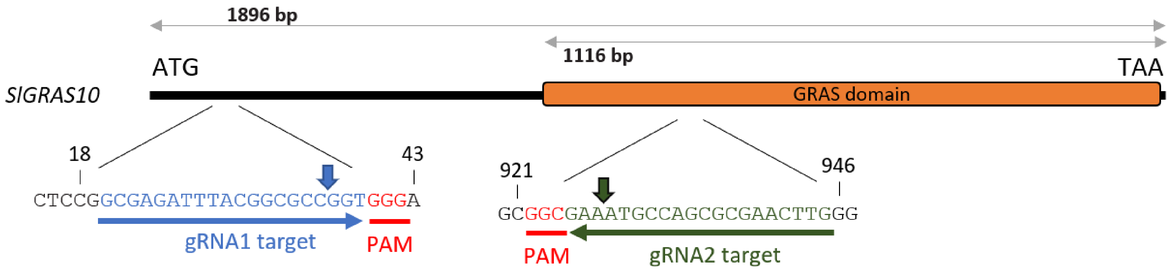
Background

The GRAS are plant-specific transcriptional factors involved in plant growth and development. However, their role in fruit development is not fully understood. Recent genome-wide analysis of the GRAS gene family in tomato showed that the gene *SlGRAS10* might be involved in tomato fruit ripening or/ and flower fertilization.

Experimental approach

Two constructs using CRISPR/Cas9 were generated for targeted mutations in two different regions of the gene.



Mutant tomato plants were obtained by performing the *Agrobacterium tumefaciens*-mediated transformation method.

Results

T_1 progeny was screened for the absence (by genetic segregation) of both *Cas9* and *NptII* (kanamycin resistance): 27% (7/23) of the plants were T-DNA free. Six of them had been mutated with gRNA2 (the most efficient guide RNA).

Sequence analysis of T_1 plants revealed 5 types of mutations

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WT   : 5′-GCCGCTTTACGGTCGCGCTTGAACC-3′
Del1: 5′-GCCGCTT-ACGGTCGCGCTTGAACC-3′
Del2: 5′-GCCGCTT--CGGTCGCGCTTGAACC-3′
Del3: 5′-GCCGCTT---GGTCGCGCTTGAACC-3′
Del4: 5′-GCCGCTT----GTCGCGCTTGAACC-3′
Ins1: 5′-GCCGCTTTTACGGTCGCGCTTGAACC-3′
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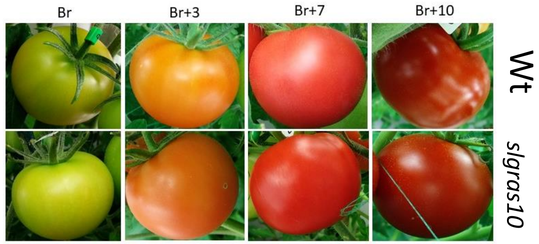
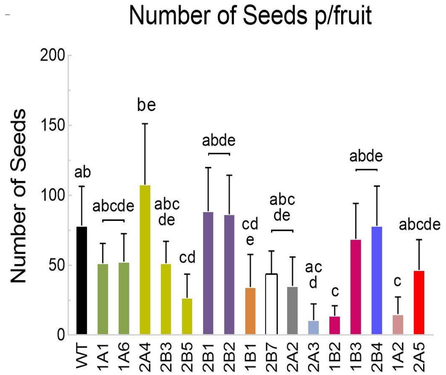


- Ripening processes were not severely influenced.
- Seeds´ number was affected in some mutated lines.
- Further analysis is necessary to elucidate the role of the *SlGRAS10* in these processes. For that, T_2 of the lines with a reduced number of seeds are being generated.



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