CRISPR/Cas9-MEDIATED KNOCKOUT OF GALACTINOL SYNTHASE-ENCODING GENES REDUCES RAFFINOSE FAMILY OLIGOSACCHARIDE LEVELS IN SOYBEAN SEEDS

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Introduction

Raffinose family oligosaccharides (RFOs) are major soluble carbohydrates in soybean seeds that cannot be digested by human and other monogastric animals. Understanding of RFOs biosynthesis and metabolism can help manipulate the amount of these carbohydrates in soybean products. In this study, we utilized a dual gRNAs CRISPR/Cas9 system to induce knockouts in two soybean galactinol synthase genes, *GmGOLS1A* and its homeolog *GmGOLS1B,* which are putatively involved in an early step of RFO biosynthesis. The CRISPR/Cas9 construct employed induced various deletions in the target sites or sequences spanning the two target sites of both *GmGOLS1A* and *GmGOLS1B* genes at T0 generation. A subset of induced alleles was successfully transferred to progeny and we identified null segregants of single and double mutant genotypes without off-target induced mutations. When grown in greenhouse condition, double mutant lines showed a reduction in the total RFO content of soybean seed by 35.2% decrease. On average the amount of stachyose, the most predominant RFO in soybean seeds, decreased by 35.4% in double mutant soybean, while the raffinose content increased by 41.7%; the amount of verbascose, a complex RFO synthesized from stachyose, was also reduced. No difference in seed germination, plant development and morphology was observed in the mutants. Our findings indicate that *GmGOLS1A* and *GmGOLS1B* contribute to soybean oligosaccharides profile through RFO biosynthesis pathways, and are promising targets for future investigation, as well as crop improvement efforts.

Experimental methods

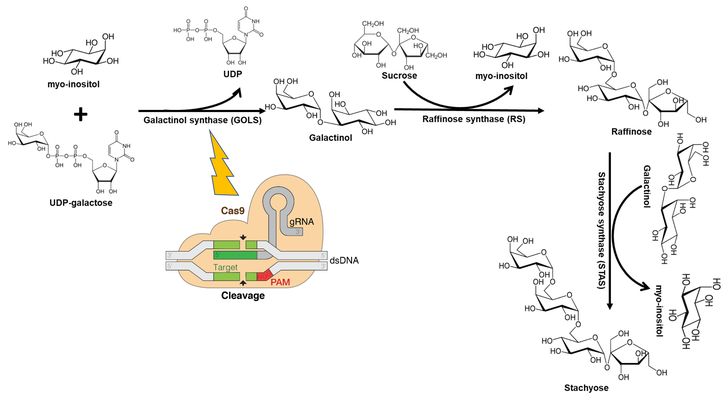

Figure 1. The biosynthesis pathway of RFOs in soybean seeds. Galactinol synthase (GOLS) – encoding genes was knocked out in this study using CRISPR/Cas9 targeted editing.

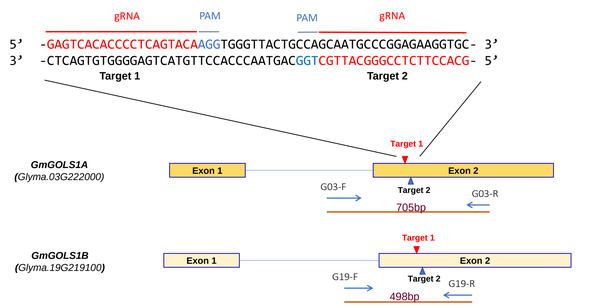

Figure 2. Complementary sequences to the guide RNAs on the targeted sites shared by *GmGOLS1A* and *GmGOLS1B*. The locations of the primer pairs used to genotype *GmGOLS1A* and *GmGOLS1B* are noted on the respective genes' diagrams.

Results

Genotyping of T0 generation

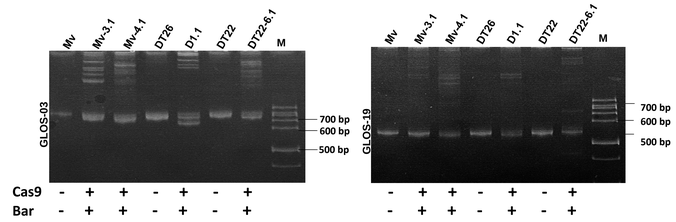

Figure 3. Early identification of edited GLOS genes at T0 generation using PAGE-based genotyping. Edited plants show heterozygosity at target sites, which can be identified as slow-moving heteroduplex bands

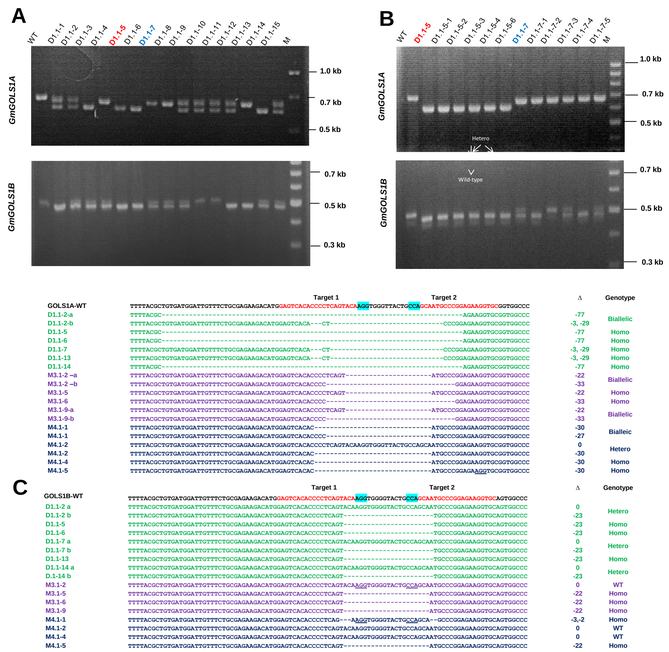

Figure 4. Gel electrophoresis shows targeted mutant segregation in T1 (A) and T2 (B) generation plants from the D1.1 line (DT26 background). In (C) are genotyping results of selected T1 plants from three T0 events D1.1, M3.1, M4.1. Δ indicates the targeted sequence changes: 0 for no change, - for deletion, + for insertion. The genotypes of T1 mutant lines were classified as biallelic for two different mutant alleles, hetero for heterozygotic with one wild type allele and one mutant allele, homo for homozygotes with two identical mutant alleles.

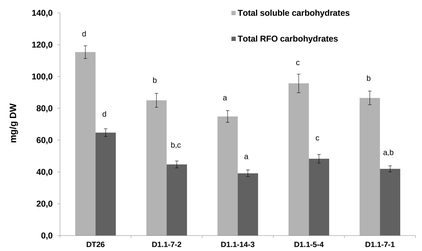

Figure 5: Total soluble carbohydrates and total RFOs as measured over dry weight by high performance liquid chromatography (HPLC). Wild-type seeds are of DT26 cultivar; D1.1-7-2, D1.1-14-3 are seeds from T2 *gmgols1A* single mutant; D1.1-5-4, D1.1-7-1 are seeds from T2 *gmgols1A gmgols1B* double mutants. Statistical analysis was done using one-way ANOVA followed by a post-hoc Tukey's multiple range test. Different letters denote significant differences at p <0.05., n=4.

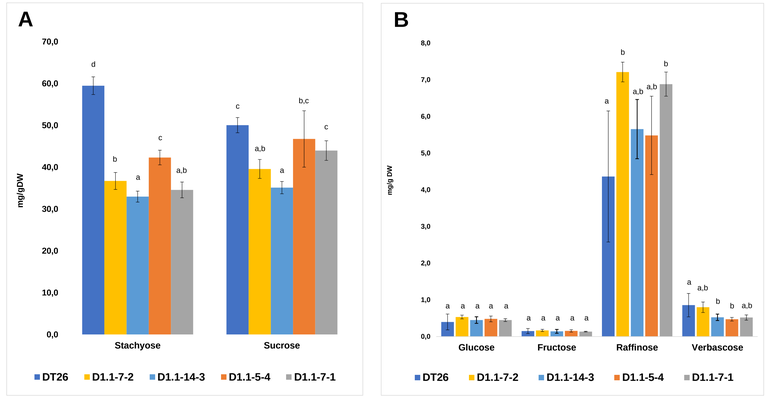

Figure 6: Carbohydrate composition in soybean seeds, with the major carbohydrates stachyose and sucrose in (A) and less abundant carbohydrates in (B). Wild-type seeds are of DT26 cultivar; D1.1-7-2, D1.1-14-3 are seeds from respectively named T2 *gmgols1A* single mutant; D1.1-5-4, D1.1-7-1 are seeds from T2 *gmgols1A gmgols1B* double mutants. Statistical analysis was done using one-way ANOVA followed by a post-hoc Tukey's multiple range test. Different letters denote significant differences at p <0.05

Conclusions

- CRISPR/Cas9 system is an efficient, fast and cost-effective approach for simultaneous targeted mutagenesis of soybean multi-copies genes.
- Successful mutagenesis of *GmGOLS1A/GmGOLS1B* led to new carbohydrate profile.
- Reduction of stachyose contributed to an overall decrease of soybean seeds RFO content.
- The modified content of sucrose and raffinose merits further investigation of galactinol synthase activity.

Future work

- Analyze single mutants of *GmGOLS1A*, *GmGOLS1B* to identify the contribution of each genes.
- Evaluate mutants' plant and seed characteristic in other growth conditions.
- More detailed analysis of soybean seed carbohydrates with regards to galactinol synthase activity.
- Investigation into remaining galactinol synthase genes in soybean, as well as other genes involved in RFO biosynthesis.

References

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