CRISPR/Cas9 Mediated knockout of Galactinol Synthase Encoding Genes Reduces Raffinose Family Oligosaccharide Levels in Soybean Seeds

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Abstract: Raffinose family oligosaccharides (RFOs) are major soluble carbohydrates in soybean seeds that cannot be digested by human and other monogastric animals. Hence, a major goal is to reduce RFO levels to improve the nutritional quality of soybean. 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*. Genotyping of T0 plants showed that the construct design was efficient in inducing various deletions in the target sites or sequences spanning the two target sites of both *GmGOLS1A* and *GmGOLS1B* genes. A subset of induced alleles was successfully transferred to progeny and, at the T2 generation, we identified null segregants of single and double mutant genotypes without off-target induced mutations. The seed carbohydrate analysis of double mutant lines showed a reduction in the total RFO content of soybean seed from 64.7 mg/g dry weight to 41.95 mg/g dry weight, a 35.2% decrease. On average the stachyose content, the most predominant RFO in soybean seeds, decreased by 35.4% in double mutant soybean, while the raffinose content increased by 41.7%. A slight decrease in verbascose content was also observed in mutant lines. Aside from changes in soluble carbohydrate content, some mutant lines also exhibited increased protein and fat contents. Otherwise, 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. Our results also demonstrate the potential in using elite soybean cultivar for transformation and targeted genome editing.

Keywords: CRISPR/Cas9; GmGOLS1A; GmGOLS1B; raffinose family oligosaccharides; soybean