ABSTRACT

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Grant Title: Genetic dissection of plant complex N-glycan functions- enigma in plant biology

Objective: The objective of this study is to identify role of complex N-glycans (CGs) unique to plants. CGs are highly processed form of oligosaccharides attached to asparagine (N) residue of eukaryotic proteins. Large number of secreted or post-ER (endoplasmic reticulum) membrane proteins are decorated with one or more CGs. CGs are essential for survival of animals, and mutations in genes involved in CG biosynthesis result in a range of phenotypes from lethality, when it is severe, to various developmental defects called CDG (congenital disorder of glycosylation). The structure of plant CGs are distinct from animal CGs and unlike animals, the model plant *Arabidopsis thaliana* can complete its life cycle even if it lacks CG biosynthesis entirely. However, CG-deficient Arabidopsis mutants were more sensitive to mild salt stress, suggesting plants developed unique function associated with CGs. To understand the unidentified functions of plant CGs, Arabidopsis mutants hypersensitive to CG deficiency were analyzed and causal genes were determined by next generation sequencing.

Methods used: Selected mutant lines were crossed with Ler-0 ecotype to generate segregating F2 populations. F2 seeds were sown on 1x Murashige and Skoog (MS) media supplemented with 4 μM KIF to identify the genome mutation. These plants were analyzed using indel markers for rough mapping. Fine mapping using Next-generation sequencing was carried out with bulk DNA of approximately 100 F2 plants selected by KIF sensitivity and PCR. The genomic DNA of 100 F2 mutant segregating pools were used to prepare DNA-seq library preparation, and the Illumina NextSeq run were performed at Texas A&M Institute of Genome Sciences & Society core facility with a read length of 150 bp paired-end. Sequencing reads were mapped to the Arabidopsis TAIR10 genome using Bowtie2 (version 2.2.4), allowing up to two mismatches. The mapping interval and candidate mutations were identified using the Galaxy platform.

Results obtained: Based on the root phenotypes, we classified the obtained mutants into three groups: class I plants showed normal root growth on MS media and showing KIF hypersensitivity; class II plants showed shorter roots than parental line without KIF and showing KIF hypersensitivity; class III plants showed swollen roots on MS with or without KIF. We focused on class I and class II and isolated 50 class I and 58 class II mutant lines, respectively. Mapping and Next generation sequencing of mutant lines revealed that causal gene candidates for 38 lines. To date, mutations in total 64 lines were mapped by PCR screening, and causal gene candidates were identified for 42 lines. This analysis showed that 31 lines were allelic to mutants previously shown to be KIF-sensitive, including cellulose synthase complex subunit *cesA6*, *fla4/sos5, cobra*, *pht4;6*, as well as *stt3a*. Identification of these mutations confirmed that our large-scale screening is in line with previous studies, reinforcing the strong linkage between CG with the function of GA and cell wall biogenesis. Candidate genes for 11 mutant lines have not been characterized previously in relationship to CGs and encoded several cell wall modification enzymes, vesicle transport proteins, and membrane transporters for hormone homeostasis. One of the identified mutations encoded a novel dominant negative allele of ARF family GTPase. The mutation caused KIF-sensitivity and multiple organelle abnormality, suggesting proper CG maturation is important for vesicle transport capacity of plant cells.