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Grant Title: Fucosyltransferases and sustainable production of high-value natural products

Abstract:
Fucosylated steroidal or triterpenoid saponins possess valuable medicinal properties. The limited industrial exploitation of these compounds is due to resource scarcity and purification challenges. Our research aimed to address three objectives: (I) discover new fucosylation enzymes for high-value plant products, (II) characterize newly discovered plant fucosyltransferases, and (III) identify enzymes involved in plant UDP-α-D-fucose biosynthesis.

We employed diverse methodologies starting by metabolomics analysis to profile metabolites from plants such as *Liriope muscari*. RNA-sequencing data was used to identify candidate genes associated with fucosylated saponins biosynthesis. We further expressed candidate genes in bacterial, yeast, and plant systems for functional assays. In these assays we tested enzyme activities, such as hydroxylation and fucosylation of steroidal saponins. Phylogenetic analysis guided our exploration of potential 2OGDDs involved in hydroxylation. Through gene co-expression analysis, we investigated correlations between metabolite levels and gene expression to pinpoint key biosynthesis genes. Finally, we conducted *in vitro* enzyme assays to synthesize UDP-α-D-fucose using bacterial enzymes.

Our results include the discovery of novel glycosyltransferases responsible for adding D-fucose to steroidal saponins, alongside enzymes involved in converting cholesterol to ruscogenin. We identified three cytochrome P450 enzymes that convert cholesterol to diosgenin. Through phylogenetic analysis, gene co-expression studies, and functional assays in *Nicotiana benthamiana* and yeast, we identified 2OGDD as the enzyme converting diosgenin to ruscogenin. Notably, it represents the first sterol C1 hydroxylase producing ruscogenin, a substrate for glycosyltransferases attaching sugars to steroidal aglycones. Furthermore, we discovered two novel glycosyltransferases attaching fucose to steroidal aglycones. Our attempts to identify enzymes involved in UDP-α-D-fucose biosynthesis were challenging. While utilizing transcriptome mining and conducting in vitro enzyme assays with bacterial enzymes from *Anoxybacillus tepidamans*, we identified potential plant genes associated with fucose biosynthesis. However, we were unable to produce UDP-α-D-fucose *In vitro.*

In conclusion, our research resulted in the discovery and characterization of enzymes involved in the biosynthesis and fucosylation of steroidal saponins, laying the groundwork for sustainable production of these high-value metabolites. However, structural characterization and complete elucidation of the UDP-α-D-fucose biosynthetic pathway remain areas for future work. These findings offer a promising approach to produce environmentally friendly alternatives to QS-21.