Understanding Natural Product Biosynthesis in Blueberry

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Blueberries (Vaccinium spp.) are widely known for their flavor and human health benefits due to the production of specialized metabolites with potential positive health aspects. Iridoids are a class of specialized metabolites in plants that have known human health benefits, including anti-inflammatory and anti-cancer properties (Dinda et al., 2011). Recent work has found that blueberries make iridoid compounds, with a subset of wild and cultivated blueberry species making the specific iridoid glycoside monotropein (Leisner et al., 2017). While the biosynthetic pathway of iridoid compounds is well studied in other plants, including the model medicinal plant Catharanthus roseus (Kellner et al., 2015), the genes involved in monotropein biosynthesis in blueberry are not known. Additionally, the human health benefits of monotropein in blueberry are not well studied. Elucidating genes in the monotropein biosynthetic pathway would not only increase our understanding of this important metabolic pathway but create avenues for future research on understanding the human health impacts of iridoid production in blueberry. For this work, the key enzyme geraniol synthase (GES) was analyzed. GES has been identified as a key enzyme associated with iridoid biosynthesis (Mint Evolutionary Genomics Consortium, 2018), catalyzing the conversion of geranyl pyrophosphate (GPP) to geraniol (Fig. 1) in the plastid during one of the earliest steps of the iridoid biosynthetic pathway. The purpose of this study was to isolate and sequence this key gene in the iridoid biosynthetic pathway and to perform functional enzyme characterization to understand and validate its specific role in iridoid production in blueberry.

To isolate GES, RNA was extracted from various tissues of the blueberry cultivar Ornablue which has been shown to produce the iridoid glycoside monotropein (Leisner et al., 2017). The extracted RNA was treated with DNase and reverse transcribed into cDNA which was used as a template for polymerase chain reaction using gene-specific detection primers. The GES gene, which was amplified in floral tissues, was purified and sent for sequencing. The resulting sequence of the amplified GES was aligned with the computationally derived sequence of GES used to design the detection primers. When aligned, the amplified GES with high-quality sequencing peaks covered 33% of the computationally derived sequence but with 95% identity, indicating its similarity to the computationally derived gene. A GES gene construct was then designed, commercially synthesized, and cloned into E. coli BL21 (DE3) competent cells for heterologous expression.

This research is ongoing, and current work is focused on expressing the GES gene in E. coli cells and optimizing the protein purification step to increase the yield of the isolated GES protein. Once purified, the protein along with its substrate GPP will be used in enzyme assays and analyzed by gas chromatography-mass spectrometry to functionally characterize the GES enzyme in blueberry. Overall, results from this study will provide new insights into the function of a key gene within the iridoid biosynthetic pathway, which will allow for future work into the function of these iridoid compounds in blueberry, and lead to a better understanding of the human health benefits of blueberries.

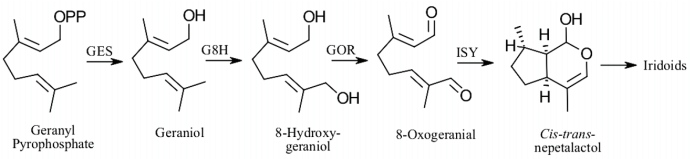

Figure 1. Early iridoid biosynthetic pathway. GES, geraniol synthase; G8H, geraniol 8-hydroxylase; GOR, 8-hydroxygeraniol oxidoreductase; ISY, iridoid synthase (Adapted from Lichman et al., 2020).

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Statement of Research Advisor

This project is part of a larger effort to identify all biosynthetic pathway genes for monotropein biosynthesis in blueberry. GES has been shown to be a key enzyme associated with plants that make iridoid compounds across a wide phylogeny of the plant kingdom. Therefore, the work done in this project provides critical information as to why some cultivars of blueberry do and do not make iridoid compounds. This paves the way to understand more about the genetic regulation of this pathway, which makes this type of natural product research more amendable to future clinical evaluation.
-Courtney P. Leisner, Biological Sciences

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Authors Biography

Emma M. Peacock received a B.S. degree in Microbial Biology (Cell/Molecular) with a minor in Mathematics at Auburn University in the spring of 2022. Starting in the fall of 2022, she will be a graduate student within the Interdisciplinary Graduate Program at Vanderbilt University. During her time at Auburn, her research was focused on identifying key genes involved in the iridoid biosynthetic pathway in blueberry.

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Dr. Courtney P. Leisner is an Assistant Professor in the Department of Biological Sciences at Auburn University. She received her B.S. from The College of William and Mary, M.Sc. in Botany from Washington State University and her Ph.D. in Plant Biology from the University of Illinois Urbana-Champaign. Following graduate school Dr. Leisner was a Ruth L. Kirschstein National Research Service Award Postdoctoral Fellow funded through the National Institute of Health. Dr. Leisner's research group focuses on climate change impacts on plant development and metabolism, with the goal to engineer a more sustainable future food supply.