**Enhancing Petunia Varieties through Advanced Genetic Techniques: Modifying Flower Colors via Gene Editing and Gene Overexpression**

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Enrolled Semester: Fall, 2023

B.S. in Biological Sciences, University of California, Davis, 2023.

**Abstract**

This report presents two innovative genetic modification strategies aimed at expanding and enhancing the color palette of petunia. Utilizing CRISPR-Cas9 technology, we target specific pigment-related genes for precise, transgene-free editing, while parallel efforts involve traditional gene overexpression to intensify and diversify floral colors. These methods support the development of enhanced petunia varieties that meet both aesthetic and commercial cultivation standards without relying on traditional approaches.

**Introduction**

Petunia is one of the most economically important ornamental plants worldwide, prized for its diverse flower colors and extended blooming period in gardens, containers and urban landscapes. Petunia contributes substantially to the horticultural sector's economic performance in the United States, representing a significant portion of the annual wholesale market for ornamental plants. According to the USDA's 2020 Floriculture Crops Summary, petunias sold in pots generated a wholesale value of over $55 million, with more than 26 million pots sold in 2020. Among petunia cultivars, elite commercial varieties are particularly notable for their vigorous growth habit and exceptional flowering performance. However, introducing additional traits of interest such as flower color into these elite commercial varieties through conventional hybridization and selection is challenging due to their highly heterozygous nature. Traditional breeding approaches risk disrupting existing desirable traits, necessitating innovative genetic strategies to enhance these varieties while preserving their superior characteristics.

Petunia flower coloration is primarily dictated by the biosynthesis of anthocyanins, a complex process involving several key enzymes and regulatory genes. This pathway includes crucial structural genes as well as various transcription factors that regulate their expression (Fig.1). Our transgene-free gene editing project aims to manipulate this pathway by targeting genes involved in regulating anthocyanin biosynthesis. For example, editing repressor-type transcription factors can enhance anthocyanin accumulation and deepen flower color. Modifying genes involved in metabolic flux can redirect precursor availability toward anthocyanin biosynthesis. Disrupting key structural genes may result in white flowers by blocking critical enzymatic steps in the pathway. Furthermore, editing genes that influence anthocyanin hydroxylation patterns can broaden the range of achievable pigment hues.

To expand the color palette of petunia flowers, we are implementing strategic gene overexpression approaches. By heterologously overexpressing anthocyanin-activating genes from snapdragon, we aim to enhance anthocyanin biosynthesis to develop purple and dark purple flower phenotypes. In parallel, overexpressing a betalain biosynthesis gene from sugar beet will produce novel bright red and dark red coloration. Additionally, enhanced expression of hydroxylation-related genes will increase delphinidin accumulation, leading to more pronounced blue flower colors. These complementary genetic engineering strategies are designed to create a diverse spectrum of flower colors, significantly enhancing both the commercial and aesthetic appeal of petunia varieties.

Recent advancements in genetic engineering, particularly the development of CRISPR/Cas9 gene-editing technology, have provided promising new methods for enhancing petunia varieties. One advantage of CRISPR genome editing over traditional genetic engineering is that transgene-free modifications can be achieved.

Several transgene-free gene editing methods have been developed. For example, Ribonucleoprotein (RNP) delivery introduces pre-assembled CRISPR/Cas complexes directly into plant cells, particle bombardment uses high-velocity particles to deliver editing components into plant tissue, and virus-based vectors employ viral particles for efficient delivery of gene-editing tools. Despite these advancements, each method has drawbacks: RNP delivery often struggles with low editing efficiency, particle bombardment can cause cell damage and inconsistent delivery, and virus-based vectors face limitations in virus-host specificity and can

trigger immune responses. Unlike other transgene-free methods, our proposal offers a novel approach with broad applicability across diverse plant species through targeted manipulation of DNA repair pathways. The pivotal component of our methodology is the strategic suppression of a specific DNA repair-related polymerase involved in alternative repair mechanisms. This enzyme plays a dual role in plant genome modification: it mediates microhomology-mediated end joining (MMEJ) during DNA repair and facilitates T-DNA integration into host genomes. By transiently suppressing this polymerase during CRISPR/Cas9-mediated genome editing, we can decrease the frequency of T-DNA insertion while maintaining efficient gene editing via the classical non-homologous end joining (NHEJ) pathway. This is particularly valuable for developing non-GMO edited crops, as it minimizes the risk of foreign DNA persistence in the plant genome.

The significance of this method is exemplified in our current work with an elite commercial petunia variety with a complex genetic background derived from multiple interspecific hybridizations. Due to its highly heterozygous nature and exclusive vegetative propagation, traditional breeding approaches are ineffective for trait improvement while maintaining its unique horticultural characteristics. Our DNA repair suppression strategy enables precise genetic modifications without transgene integration, preserving the plant's non-GMO status. This not only satisfies regulatory requirements but also addresses market demands for non-GMO ornamental varieties while allowing for targeted trait enhancement. Furthermore, this methodology can be adapted for other vegetatively propagated crops where maintaining genetic fidelity while introducing beneficial traits is crucial.

**Objectives**

The goal of this project is to modify the flower color through genetic engineering approaches. Three objectives are proposed to achieve this goal.

- To Modify Flower Color by Editing Structural Genes in Anthocyanin Pathway without Transgene Integration

- To Intensify Flower Pigmentation by Knocking Out Transcription Factors That Act as Repressors in the Anthocyanin Biosynthesis Pathway

- To Diversify Flower Coloration by Overexpressing Key Genes Involved in Pigment Production

**Research Progress**

For the first objective, we have constructed vectors for editing key structural genes and have successfully transformed model petunia cultivars. We are currently monitoring for phenotypic changes. Vectors targeting additional structural genes are under construction.

For the second objective, vectors targeting anthocyanin repressor genes have been constructed and transformed into model cultivars. Observations are ongoing.

For the third objective, we have transformed overexpression vectors carrying pigment-activating genes into model cultivars. Enhanced pigmentation has been observed, demonstrating success in color diversification.

**Educational Achievements and Coursework**

I obtained my bachelor's degree from the University of California, Davis, graduating with the highest honors and a GPA of 3.98 in Biological Sciences. This strong foundation has been vital in preparing me for advanced scientific inquiries and research.

At the University of Florida, I have furthered my education by completing several specialized courses that directly support my research in genetic engineering of horticultural crops. These courses include:

- PLS5222C (30398) - Propagation of Horticultural Crops
- AGR5307 (24456) - Molecular Genetics of Crop Improvement
- PCB5530 (16626) - Plant Molecular Biology and Genomics
- STA6093 (16790) - Introduction to Applied Statistics

I am currently enrolled in PLS5222C and maintain a 4.0 GPA, continuously applying learned concepts to my ongoing research on modifying flower coloration in petunia using advanced gene-editing technologies.

**Timeline and Future Directions**

I began my PhD studies in horticultural genetics at the University of Florida in August 2023, with an anticipated graduation date in the summer of 2028. My qualifying exam is expected to schedule in the summer of 2025. Over the next few years, my research will concentrate on advancing plant biotechnology to enhance color variations in petunia. This timeline is designed to efficiently manage my research goals and academic requirements, leading up to the preparation and defense of my dissertation in 2028.

## Figures

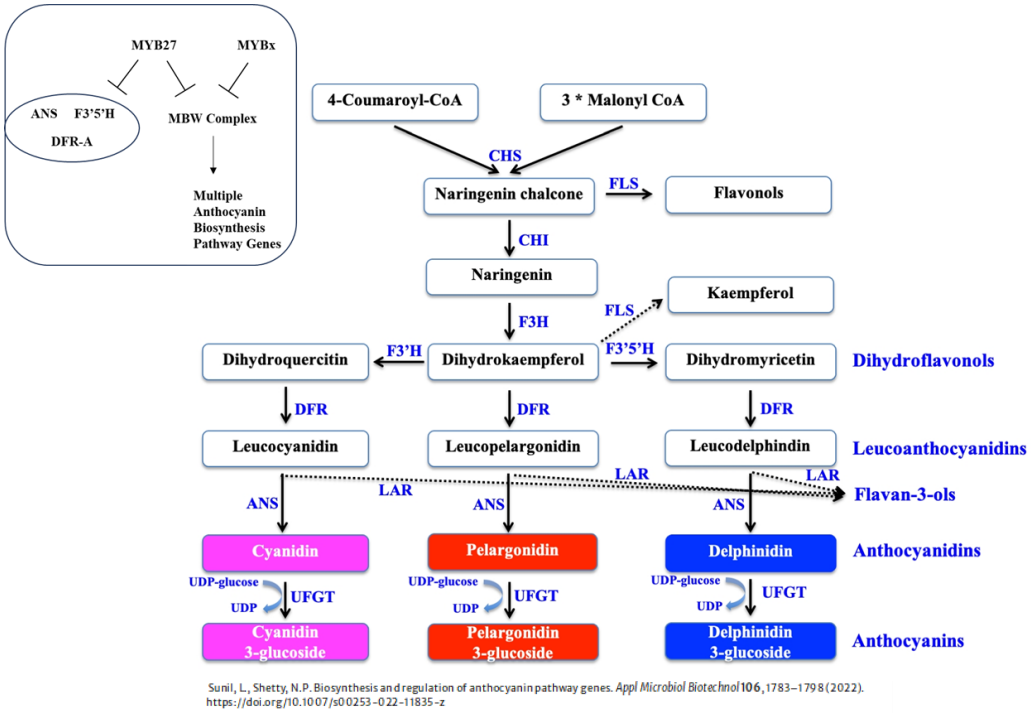


Fig. 1, Biosynthetic pathways of anthocyanins. CHS, chalcone synthase; FLS, flavonol synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′,5′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; UFGT, flavonoid 3-O-glucosyltransferase; UDP-glucose, uridine diphosphate glucose; asterisk indicates multiplication (Sunil et al., 2022). MYB repressors may directly limit the expression of structural genes and disrupt the formation of the MBW complex, thereby repressing anthocyanin biosynthesis.

Fig. 2, Comparative display of Petunia flowers showing the wild type (left) with a standard pink hue and a genetically modified variant (right) with a deeper pink color due to the overexpression of a key structural gene.

## References

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