Chemical Analysis of Dihydrochalcones within *Malus* leaves

Samantha Page[1,2], Ben Gutierrez[2], Victoria Meakem[2]

[1]Hobart and William Smith Colleges, 300 Pulteney St, Geneva, NY 14456
[2]USDA-ARS Plant Genetic Resources Unit, Geneva, NY 14456

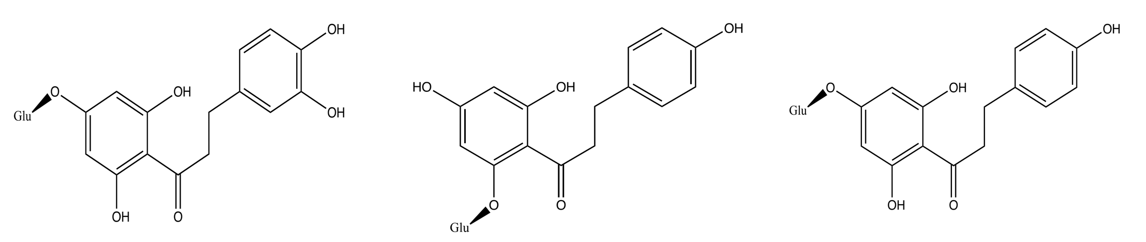
Introduction

Apple trees may offer more than the sweet fruit that ripen each year during the fall season. Dihydrochalcones (DHCs), such as sieboldin, phloridzin, trilobatin are complex organic compounds identified in the leaves of *Malus* species[1]. DHCs are commonly known for their proposed health benefits, which include but are not limited to; anti-cancer, antioxidant, and anti-diabetic properties[2]. Depending on the origin of *Malus,* the phenotypic profiles of DHCs present differently. This creates opportunities to study genetic variation and inheritance through crossbreeding. Two heterozygous populations (containing all three DHC's) of *Malus domestica* and *Malus prunifolia* were crossed to yield three offspring populations. Predicted phenotypes were determined through known genotypes of parent *Malus* species. The seven possible phenotypes are:

- Sieboldin, Phloridzin, Trilobatin **(SPT)**
- Sieboldin & Phloridzin **(SP)**
- Sieboldin **(S)**
- Trilobatin **(T)**
- Phloridzin & Trilobatin **(PT)**
- Sieboldin & Trilobatin **(ST)**
- Phloridzin **(P)**

***Malus domestica* leaves**
SsPpTt

***Malus prunifolia* leaves**
SsPpTt



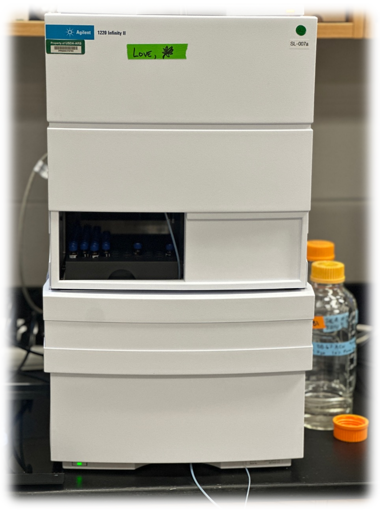
Sieboldin (S) **Phloridzin (P)** **Trilobatin (T)**

Materials & Methods

- 4-6 leaves were obtained from approximately 180 apple seedlings through the USDA PGRU greenhouse, located in Geneva, NY.
- The MiniG Homogenizer was used to grind leaves into a fine powder. Six samples were used for each cycle which lasted 45 seconds each.
- 25 mg of the ground leaf sample was combined with a 70% methanol & 2% formic acid solution. The samples were placed into the ThermoMixer for a 20-minute cycle at 1500 rpms.
- The samples were then placed directly into the Centrifuge for a 15-minute cycle at 15000 rpms.
- Lastly, 1 mL of supernatant was extracted from each sample and filtered into small vials. The vials were placed into the HPLC where a diode array detector at 280 nanometers identified DHCs within each sample.

MiniG Homogenizer

Eppendorf Centrifuge & ThermoMixer



High Performance Liquid Chromatography (HPLC)

Results & Discussion

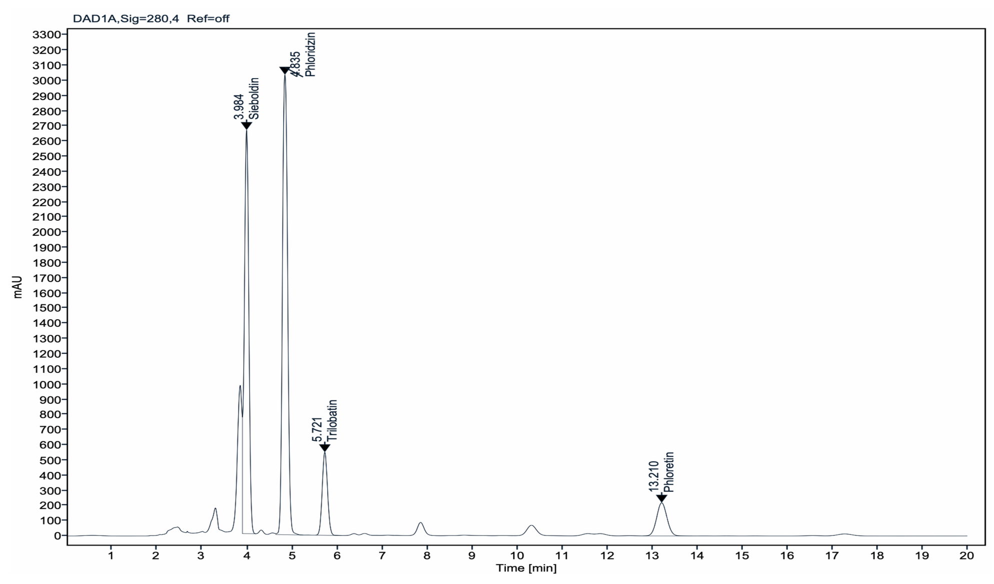


Figure 1. Chromatograph of dihydrochalcone identification. All three DHC's are present indicating this germplasm has an SPT genotype.

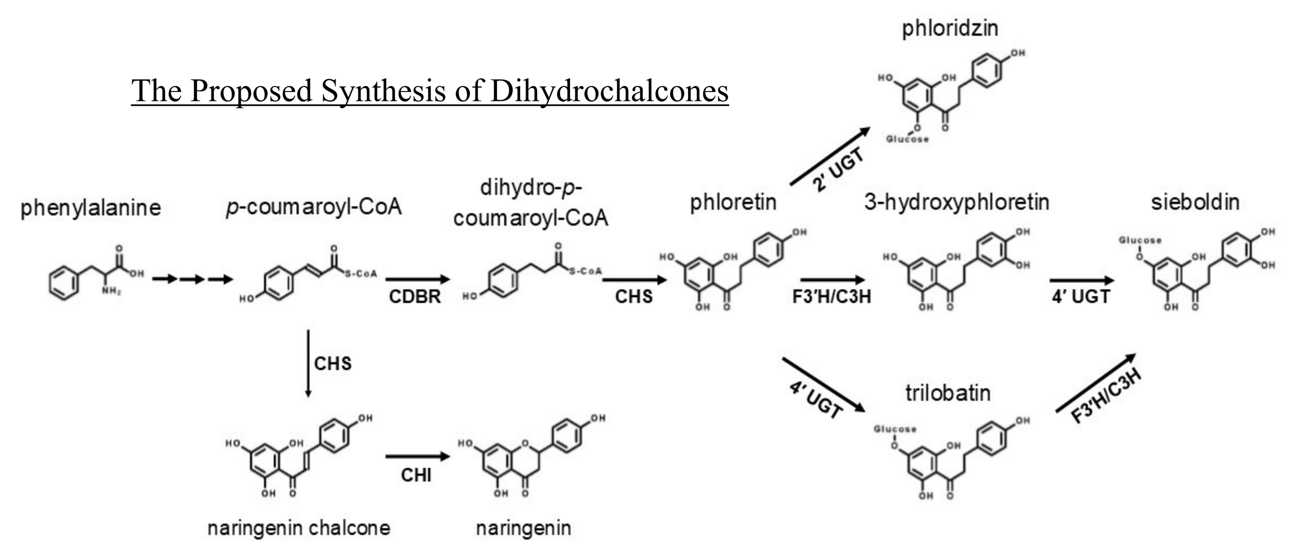


Figure 2. Both phloridzin and trilobatin are derivatives of phloretin and sieboldin is a derivative of trilobatin. This indicates that sieboldin is dependent upon trilobatin. Since sieboldin cannot exist by itself with phloridzin, SP phenotypes present as P phenotypes.

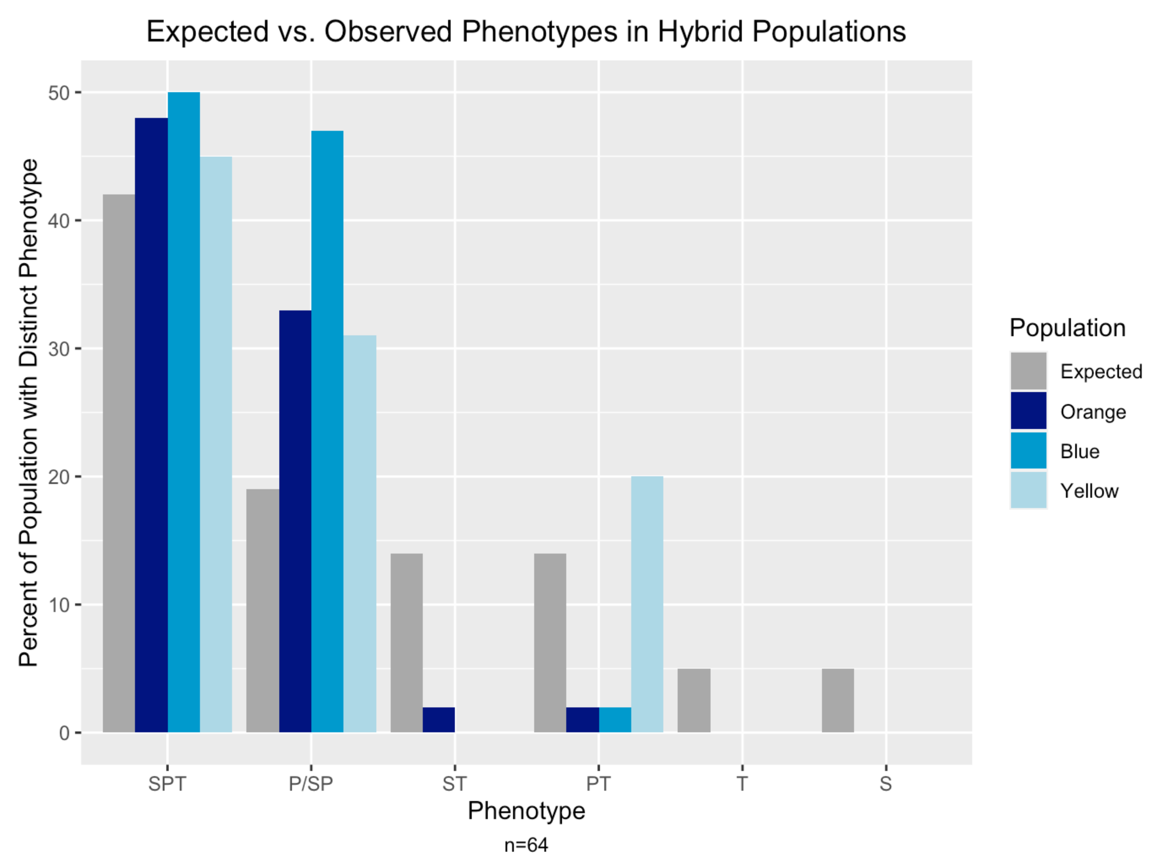


Figure 3. The expected ratios were determined through mendelian inheritance assuming three independent genes. The expected ratios are then compared to the actual phenotypes found in each separate population.

- The SPT and P/SP phenotype was more prevalent in all three populations than what was originally expected.
- The expected ST phenotype is 14%. However, 2% of the orange population and 0% of yellow and blue presented an ST phenotype.
- The expected PT phenotype is 14%. Both orange and blue only had 2%. The yellow population had 20%.
- The expected S and T phenotypes are 5%. 0% of orange, blue, and yellow populations had these phenotypes.

- **Chi-Squared analysis produces a p-value less than the alpha-value (0.05), indicating DHC's are unable to segregate and obey inheritance.**

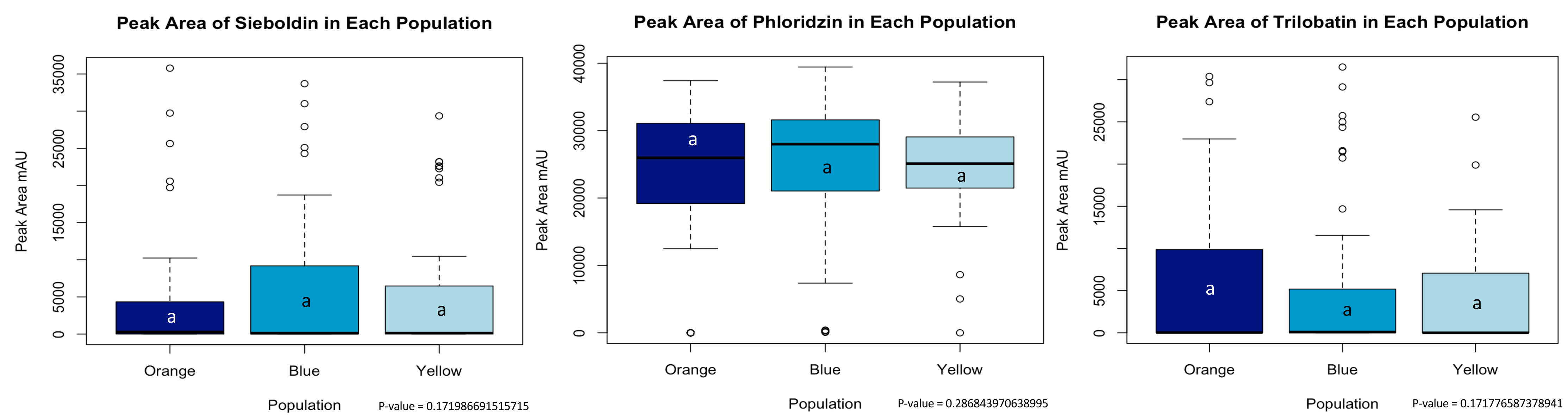


Figure 4. Box plots showing the peak area of each dihydrochalcone within the three populations. Compact letter display (CLD) indicates significance.

- Sieboldin, phloridzin, and trilobatin are similar to each other with few outliers.
- There are no statistical differences among each dihydrochalcone and population. All p-values for each dihydrochalcone were greater than the standard-alpha value (0.05).
- Sieboldin and trilobatin are similar to each other and have the same p-value. From the proposed synthesis diagram, this further illustrates the relationship between siebolding and trilobatin.
- Phloridzin dominates sieboldin and trilobatin in terms of its average peak area.

Conclusion

This study provides valuable insights into the prevalence and distribution of dihydrochalcone phenotypes within three populations. The unexpected patterns observed in the phenotypic distribution, as well as the lack of statistical differences among the dihydrochalcones and populations, pose interesting questions for future research. Interestingly, past research with different populations of *Malus* have portrayed positive findings of DHC segregation and direct correlations between DHC's[3]. This contradicts the results within the studied populations. Further investigations are warranted to interpret the underlying mechanisms of segregation of DHC's in *Malus* species.

References

[1]Stompor, M., Broda, D., & Bajek-Bil, A. (2019). Dihydrochalcones: Methods of Acquisition and Pharmacological Properties-A First Systematic Review. *Molecules (Basel, Switzerland)*, *24*(24), 4468. https://doi.org/10.3390/molecules24244468

[2]Gutierrez, B.L., Zhong, GY. & Brown, S.K. Genetic diversity of dihydrochalcone content in *Malus* germplasm. *Genet Resour Crop Evol* **65**, 1485–1502 (2018). https://doi.org/10.1007/s10722-018-0632-7

[3]Gutierrez, B.L., Arro, J., Zhong, GY. *et al.* Linkage and association analysis of dihydrochalcones phloridzin, sieboldin, and trilobatin in *Malus*. *Tree Genetics & Genomes* **14**, 91 (2018). https://doi.org/10.1007/s11295-018-1304-7

Acknowledgements

I would like to extend my gratitude to the following mentors, without whom this project would not have been possible: Ben Gutierrez and Victoria Meakem.
I would also like to thank my peers, Abe Porschet, Marcella Vennettozzi, Anna Cohen, and Jonny Aponte, for their support.