Investigating Dihydrochalcone Phenotypes and Corresponding Genotypes in Various Apple Species

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Introduction

Dihydrochalcones (DHCs) including phloridzin, sieboldin, and trilobatin have unique chemical properties responsible for both the commercial and the nutritional value of apples and have been examined as having both antioxidant[1] and anti-diabetic properties.[2] These compounds, previously measured through HPLC, are known to be expressed in five phenotypes:

1. Phloridzin (P)
2. Sieboldin and Trilobatin (ST)
3. Phloridzin and Trilobatin (PT)
4. Sieboldin, Phloridzin, and Trilobatin(SPT)
5. Trilobatin (T)

The original mapping was done through a SPTxP cross. Through this cross, a new phenotype class, PT, occurred, showing that genes coding for sieboldin migrate independent of phloridzin and trilobatin following expected Mendelian inheritance **(Figure 2.)** By analyzing the the offspring, loci for these trilobatin and sieboldin were discovered on apple linkage group (LG) 7 and 8 **(Figure 3.).** Within those loci, a gene for a chalcone 3-hydroxylase gene was identified and hypothesized to control sieboldin expression. Using a more diverse collection, consisting of ten different species, we hope to test this marker and determine if the identified gene is still tightly linked to sieboldin expression.

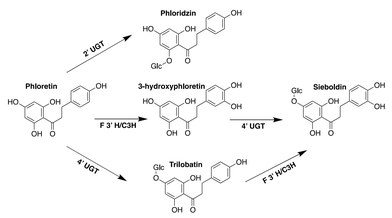


Figure 1. Proposed Dihydrochalcone synthesis

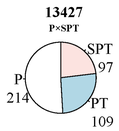


Figure 2. Observed phenotypes of a P and SPT cross.

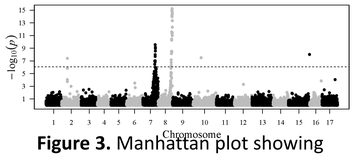


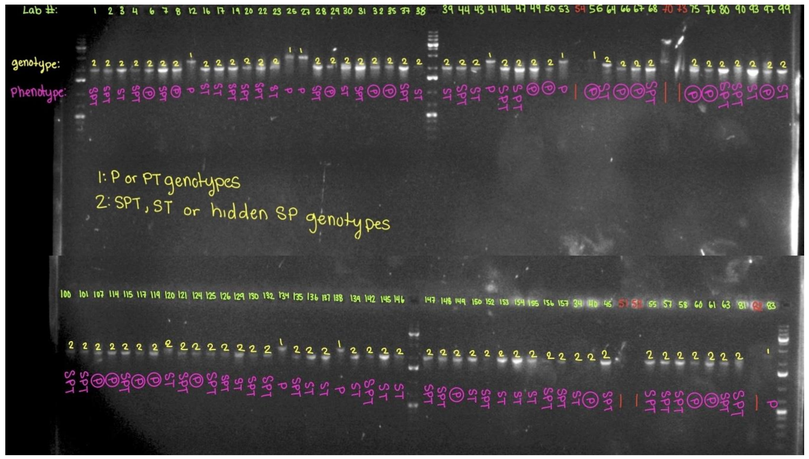
Figure 3. Manhattan plot showing activity on both LG7 and LG8

Methods

- Leaf collection: USDA Collection blocks, McCarthy Farm in Geneva, NY, "Youngest" leaf in 2.0 ml tube w/ small ball bearings, Freeze dried for 2 days
- DNA extraction: *Soltis Lab CTAB DNA Extraction Protocol*
  - preferred over a DNA extraction kit because it is known to produce larger concentrations of DNA
- PCR: 94C 15 sec, 60C 15 sec, 68C 1.15 min
- Gel Electrophoresis: 100V, 1.5 hours.
- Gel Imaging: SYBR Safe dye, UV light
- HPLC to determine phenotypes of the samples.

Results

- The genotypes, when compared to the phenotypes, showed 100% correlation **(Table 1.).** Except for two samples. It is very likely that the inconsistent samples (70 & 82) was caused by human error and should be collected again.
  - These samples are reflected by the lower percentages in both the Floribunda and Micromalus collections.
- Many accessions, such as samples 6 and 8 in **Gel 1.,** showed genotypes consistent with sieboldin expression but showed no sieboldin expression in the phenotype, even after the chemistry was verified through HPLC. These phenotypes show that the SP genotype exists though the SP phenotype is not observed **(Figure 4).**
- Red samples on **Gel 1.,** showed no bands, suggesting that there was low DNA concentrations in the samples.



Gel 1. Samples collected mostly from the B9 and E7 collection blocks. Known Phenotypes are shown in purple, where the variance in genotypes is shown in yellow. "1" typically correlates with a P or PT genotype where "2" typically correlates with a SPT ST or unique "hidden" SP. Lab number is a unique number given to each accession, red lab numbers show samples with no bands that were to be ran again on a later gel.

Species	Number of trees screened	Phenotypes Observed	Number of Trees	Genotype Correlation
Arnoldiana	2	SPT	2	100%
Atrosanguinea	2	SPT	2	100%
Floribunda	9	SPT	9	89%
		(s)P	1	100%
Micromalus	16	SPT	3	100%
		ST	7	86%
		P	3	100%
		(s)P	4	100%
Prunifolia	37	SPT	11	100%
		P	6	100%
		(s)P	20	100%
Purpurea	3	SPT	1	100%
		P	1	100%
		(s)P	1	100%
Sargentii	19	SPT	11	100%
		ST	8	100%
Sublobata	4	SPT	3	100%
		ST	1	100%
Toringo		SPT	13	100%
		ST	10	100%
		(s)P	1	100%
Zumi	3	SPT	1	100%
		ST	2	100%

Table 1. Various species samples, the phenotypes observed, and the correlation between the genotype and phenotype. The Floribunda SPT collection contained number 70 and the Micromalus ST collection contained number 86 resulting in the lower percentages.

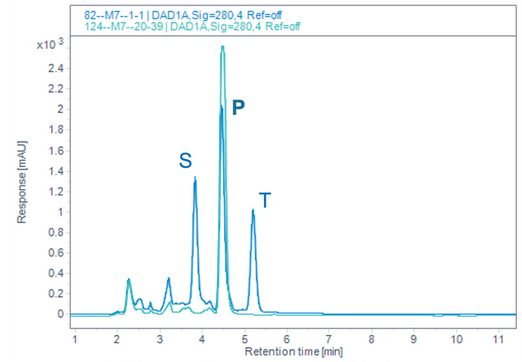


Figure 4. Chromatogram of "Hidden" SP type number 124 (Green) overlayed a chromatogram of a known SPT type (Blue). No sieboldin is expressed regardless of the genotype.

Conclusions

- Based on my collection we have confidence in our candidate gene
  - All the phenotypes could be explained by the genotypes except for the "hidden" SP types and number 82 **(Figure 5.).**
    - Number 82 should be re-collected to ensure that it was influenced by human error.
    - More of the Diversity collecting should be investigated to confirm.
- It is possible for "Hidden" SP types to exist where they have the genes coding for sieboldin expression, but do not express it.
  - "Hidden" SP types were verified through HPLC
  - A potential hypothesis is that sieboldin expression requires the presence of trilobatin.

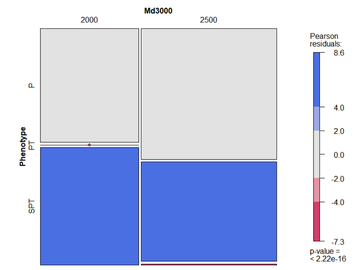


Figure 5. Mosaic plot of population phenotypes associated with Md3000 marker. Blue indicates more than expected, and red indicates less than expected assuming independence.

References

1. Gutierrez, B.L.; Arro, j.; Zhong, GY.; Brown, S.K. *Tree Genetics & Genomes,* **2018,** *14,* 19.
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3. Gutierrez, B.L.; Zhong, GY.; Brown, S.K. *Genet. Resour. Crop Evol.* **2018,** *65*, 1485-1502.
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