Determing the Genetic Determinants of Walnut Pellicle (Seed Coat) Pigmentation

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Introduction

English Walnut (*Juglans regia*) is a significant California agricultural commodity, with annual production valued at $1.84 billion (2014). A key factor in consumer acceptance and walnut export value is pellicle (seed coat) color. Recent studies have demonstrated that pellicle color is influenced by phenolic compounds, a class of compounds that includes tannins, anthocyanins, melanins, lignins and monophenols.

To identify biosynthetic pathways and metabolites that determine pellicle color, pellicles were isolated over one growing season from 5 cultivars presenting unique pellicle colors (pale, tan, red, orange, brown). Total RNA was extracted and barcoded; cDNA libraries were sequenced (SE, 50bp) using an Illumina HiSeq 3000. Metabolites were extracted and separated via GC (small, primary metabolites) or HPLC (large, secondary metabolites) and identified via MS/MS.

Our results implicate phenolic metabolites and specific, related genes in controlling cultivar-specific pellicle color. To enable construction of breeding markers, DNA from these cultivars has been included in a SNP profiling chip. Implicated genes will be evaluated for predictive power across a panel of 1500 individuals to correlate candidate genes and gene variants with pellicle phenotype.
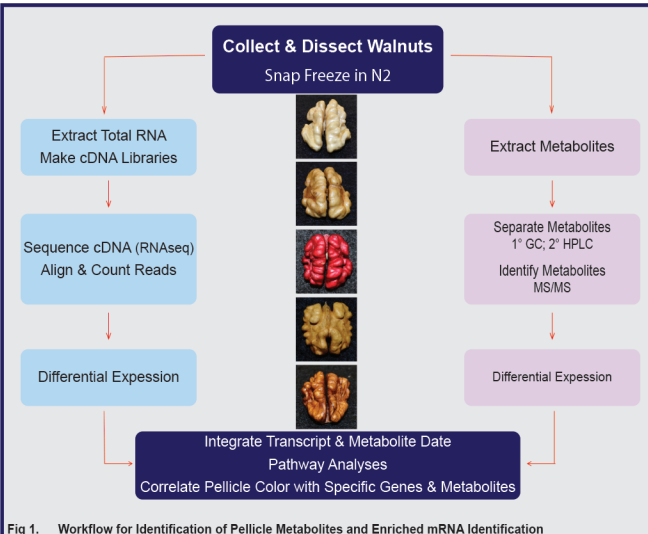

Fig 1.   Workflow for Identification of Pellicle Metabolites and Enriched mRNA Identification
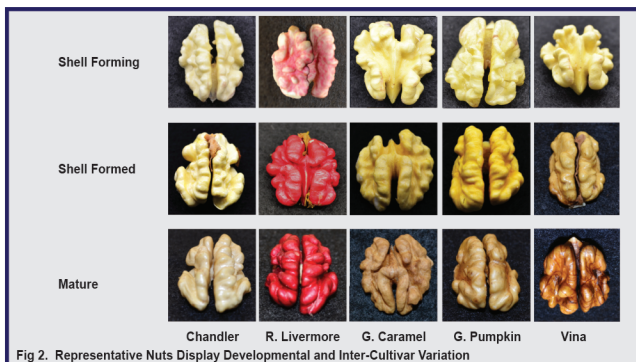
Results

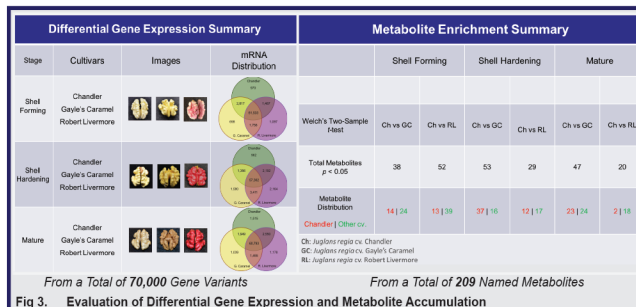Fig 2. Representative Nuts Display Developmental and Inter-Cultivar Variation

Fig 3.   Evaluation of Differential Gene Expression and Metabolite Accumulation

From a Total of 70,000 Gene Variants   *From a Total of 209 Named Metabolites*
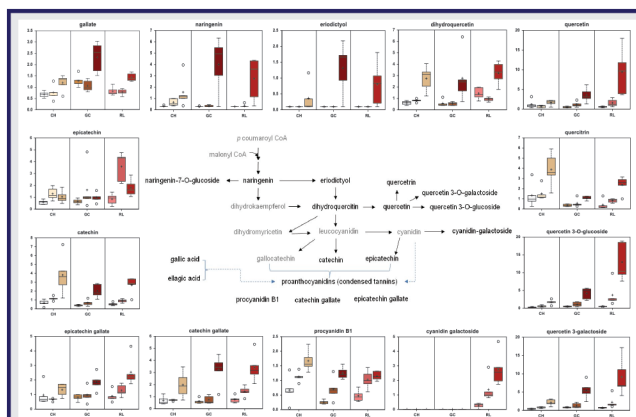

Fig 4.   Enrichment in Flavonoid Species is Largely Cultivar-Specific
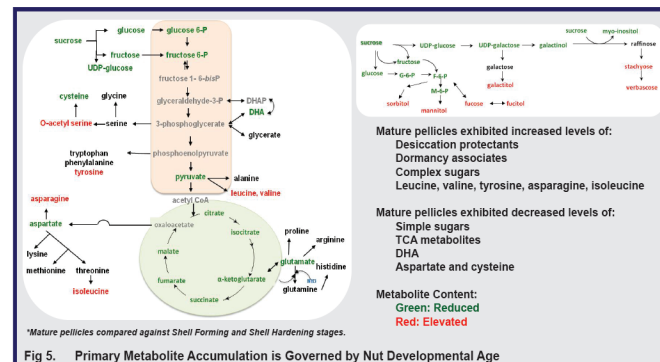Pale Pellicles Exhibit Lower Levels of Most Flavonoids

Fig 5.   Primary Metabolite Accumulation is Governed by Nut Developmental Age
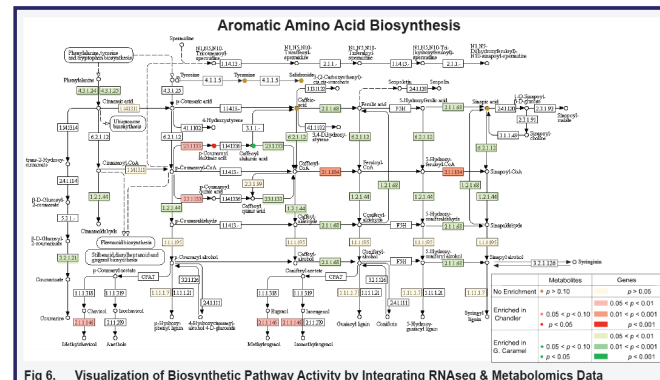
Mature pellicles exhibited increased levels of:
Desiccation protectants
Dormancy associates
Complex sugars
Leucine, valine, tyrosine, asparagine, isoleucine

Mature pellicles exhibited decreased levels of:
Simple sugars
TCA metabolites
DHA
Aspartate and cysteine

Metabolite Content:
Green: Reduced
Red: Elevated

*Mature pellicles compared against Shell Forming and Shell Hardening stages.

Aromatic Amino Acid Biosynthesis

Fig 6.   Visualization of Biosynthetic Pathway Activity by Integrating RNAseq & Metabolomics Data

Conclusions

- Pellicle primary metabolism is predominantely a function of maturity
  - Reduction in simple sugar, sugar phosphates, TCA cycle metabolites and UDP-hexoses
  - Increased levels of specific amino acids, and complex sugars

- Phenol metabolism is enriched in darker pellicles
  - e.g. naringenin, gallate, gentisate, tryptamine (all known to be pigmented)

- The dominant pigment in Robert Livermore's is an anthocyanin
  - cyanidin 3-O-galactose

- Evaluating the power of SNPs in key genes identified in this study to predict pellicle color in a panel of 1500 phenotyped and SNP typed trees will enable generation of breeding markers