Identifying the Genetic Determinants of Pellicle Coloration in Walnut via Metabolomics and RNAseq

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English Walnut (*J. regia)* is one of California's most significant agricultural commodities, with annual production valued $1.84 B (2014); a key factor for walnut exports is pellicle (seed coat) color. The walnut pellicle is rich in phenolic compounds, (incl. flavonoids, anthocyanins, tannins) which influence pellicle color. Pellicles isolated from five cultivars presenting unique pellicle colors (pale, tan, brown, orange, red) at three developmental time points (tip hardening, shell formed, mature) were used to study phenolic metabolite biosynthesis and pathway regulation during nut maturation. Pellicle samples were split into three fractions: 1.) Metabolomics by GC/MS (small, 1° metabolites) and HPLC/MS (large, 2° metabolites) 2.) RNAseq analysis (Illumina HiSeq 3000) and 3.) qPCR confirmations. Metabolomics revealed primary metabolite profiles vary by developmental time while secondary metabolite profiles were primarily influenced by cultivar. Most of the named secondary metabolites were phenols, many of which are known pigments. Global gene expression was captured via RNAseq using barcoded samples. Reads were mapped to the *J. regia* transcriptome, expressed genes reconstructed and evaluated for sample enrichment. Global RNAseq analysis revealed a developmental partition of gene expression. Expression within the phenol metabolism pathways comported with metabolomics results, confirming variety influences expression in these pathways. Confirmation of expression is being achieved via qPCR; cloning and protein activity assays are being used to confirm predicted metabolic activity of specific genes. Evaluated cultivars have been included in a genotyping project to identify candidate gene-associated SNPs, enabling marker construction and evaluation in the UC Davis Walnut Breeding Program.