FINAL REPORT **PROPOSED DURATION**: 2 years

Project Title: Functional genomics and marker development for apple sensory qualities

PI: Yanmin Zhu
Organization: USDA, ARS, Tree Fruit Research Lab
Telephone/email: 509-664-2280 ext 215 zhu@tfrl.ars.usda.gov.
Address: 1104 N. Western Ave. Wenatchee, WA 98801

Co-PI: James Mattheis
Organization: USDA, ARS, Tree Fruit Research Lab
Telephone/email: 509-664-2280 ext 249 mattheis@tfrl.ars.usda.gov
Address: 1104 N. Western Ave. Wenatchee, WA 98801

Co-PI: Bruce Barritt
Organization: Tree Fruit Research and Extension Center, WSU
Telephone/email: 509-663-8181 etaplz@wsu.edu

Collaborator: Cameron Peace,
Organization: Department of Horticulture and Landscape Architecture, WSU
Telephone/email: 509-335-6899 cpeace@wsu.edu

Budget 1:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist	
Telephone:(510) 559-6019		**Email:**cwmyers@pw.ars.usda.gov	
Item	**2007**	**2008**	
Salaries	33,000	33,000	
Benefits	10,000	10,000	
Wages			
Benefits			
Equipment			
Supplies	10,000	10,000	
Travel	1,500	1,500	
Miscellaneous	500	500	
Total	$55,000	$55,000	

The **salaries and benefits** are for hiring a postdoc dedicated to this project.
The **supplies** include common reagent for molecular genetics study and gene profiling analysis.
The budget for **travel** includes the cost for visiting Malus germplasm repository at Geneva, New York, for identify the phenotypic extremes on related fruit quality.

OBJECTIVES

1. Continue to apply the tested ethylene molecular markers for ACS1 and ACO1 in segregation populations in the WSU Apple Breeding Program to select for low ethylene production.
2. Test and apply a reported apple fruit peel red color marker in the existing WSU segregation population for selection of red color development capacity.
3. Identify potential candidate genes regulating apple firmness and crispness.
4. Elucidate relationships between expression of apple AAT (alcohol acyl transferase) genes and cultivar differences in volatile ester production.

SIGNIFICANT FINDINGS

1. Tests of two functional DNA markers related to apple climacteric ethylene production in elite breeding parents and advanced selections revealed a close relationship between these markers and fruit firmness.
2. More than 3500 seedlings in WSU breeding program have been genotyped for their allelotypes of both climacteric ethylene biosynthesis genes. The genotype data can be used by for the selection process.
3. Test of a published apple fruit skin color marker among cultivars, and two cross populations revealed a good but less-than-perfect correlation between this marker and apple fruit skin color phenotype.
4. The expression patterns of the AAT gene family were found to be associated with the phenotypic features of aromatic volatile ester generation in two cultivars with extreme aroma production phenotypes.
5. Several functional groups of genes including a short list of cell wall modifying genes and hormone metabolism related genes were identified by a large scale gene expression profiling analysis.. Microscopic examination of fruit revealed a distinguishable feature of cell wall thickness for two cultivars with distinct texture attributes.

RESULTS AND DISCUSSION

1. Test and application of DNA markers for apple ethylene biosynthesis genes Md-ACS1 and Md-ACO1 in WSU breeding parents and suitability for marker-assisted selection. (Collaborators: Dr. Cameron Peace and Bruce Barritt)

Fruit ethylene production genotypes for Md-ACS1 and Md-ACO1 were determined for 60 apple cultivars and 35 advanced breeding selections. Two alleles for each gene are commonly found in cultivated apple. ACO1 plays a minor role compared to ACS1, with homozygous ACO1-1 having lower ethylene production. In this study, ACS1-2 and ACO1-1 homozygotes had firmer fruit at harvest and after 60 days of 32-33^{o}F cold storage compared to other genotypes (Figure 1). This genotype, ACS1-2/2 and ACO1-1/1, was observed for 7 of 95 cultivars/selections including ‘Fuji’, ‘Pacific Beauty’, ‘Sabina’ and 4 breeding selections. Cultivars/selections that were homozygous ACS1-2 but not ACO1-1 were: ‘Ambrosia’, ‘Aurora Golden Gala’, ‘CrimsonCrisp’, ‘Gala’, ‘GoldRush’, ‘Huaguan’, ‘Pacific Rose’, ‘Pacific Queen’, ‘Pinova’, ‘Sansa’, ‘Sonja’, ‘Sundance’, ‘Zestar’ and 17 breeding selections. Cultivars with the heterozygous ACS1-1/2 genotype were ‘Arlet’, ‘Braeburn’, ‘Cameo’, ‘Delicious’, ‘Delorgue’, ‘Empire’, ‘Enterprise’, ‘Ginger Gold’, ‘Golden Delicious’, ‘Granny Smith’, ‘Honeycrisp’, ‘Orin’, ‘Pink Lady’, ‘Silken’, ‘Suncrisp’, ‘Sundowner’, ‘Sunrise’ and 11 breeding selections. No cultivars were detected homozygous for both ACS1-1 and ACO1-1, or for both ACS1-2 and ACO1-2. This study is the first large scale allelic genotyping of both ethylene synthesis genes for a comprehensive set of apple breeding parents used in an ongoing breeding project. The data reported here are important for informative selection of parent combinations and marker-assisted selection of progeny for breeding low

ethylene-producing apple cultivars, which are essential for better storability and improved consumer acceptance.

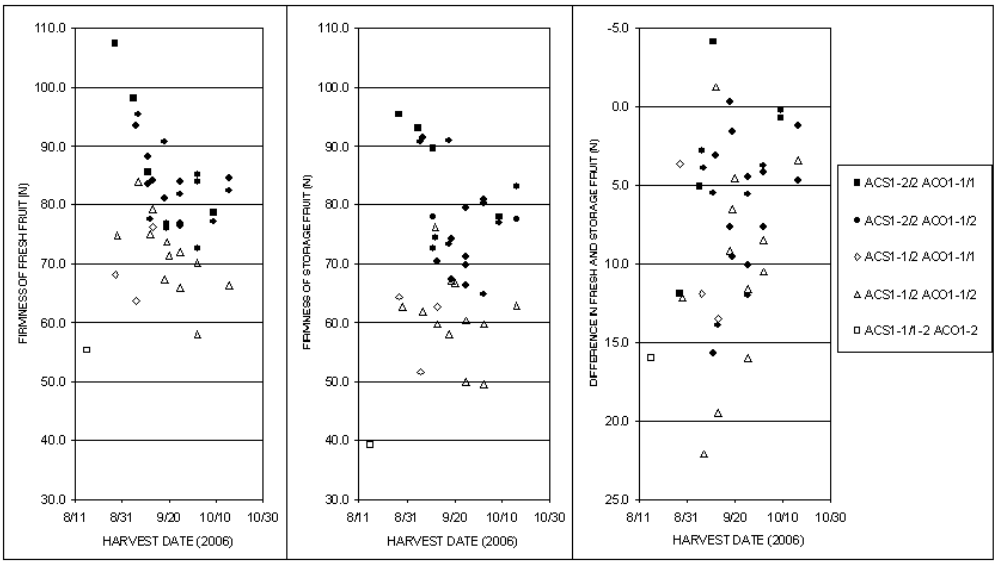

Figure 1. Fresh fruit firmness at harvest (left panel), firmness after 60 days of cold storage (middle panel) and the difference in firmness (fresh minus stored fruit) (right panel) for 40 samples displayed by their ACS1 and ACO1 genotypes across harvest dates.

What this study means to industry:
(1). Among 35 advanced selections from the WSU Apple Breeding Program, a good correlation between fruit firmness and beneficial (low ethylene production, solid symbols in above figure) genotype was observed. None of the advance selections has the otherwise high level ethylene production genotypes (empty symbols in above figure). Therefore, this result supports the implementation of maker-assisted selection to eliminate the high ethylene producer at the early stage of breeding process.

(2). The data reported here are also important for informative selection of parent combinations to produce desirable combination of ethylene production genotypes.

(3). More than 3,000 seedlings from the current breeding pipeline have been genotyped for ethylene production potential through collaboration with Dr. Peace.

2. Utility testing of MdMYB, an apple skin color marker, in two progenies
(Collaborators: Cameron Peace and Kate Evans)

A reported allele-specific dCAPS (derived Cleaved Amplified Polymorphic Sequence) marker, within the gene for the anthocyanin pathway transcription factor *MdMYB1*, associated with apple fruit skin color, was tested in 17 elite breeding parents and two apple seedling progenies. As shown in two tables below, in both progenies, the red skin color phenotype was usually associated with the *MdMYB1-1* allele. This dCAPS marker provided approximately 80% predictability in a 'Golden Delicious' × 'Arlet' and a 'Honeycrisp' × 'Cripps' Pink' progeny. Other potential genetic co-regulators may explain the less-than-perfect association. The specific dCAPS bands associated with red skin for the latter population were not the same as in the former population or previous reports, and indicates that skin color genotyping based on this marker will require prior association between specific marker alleles and color phenotypes for any

given cross. The current form of this marker could be a useful tool for apple marker-assisted breeding, particularly where 'Golden Delicious' is a parent.

Table 1. Association of *MdMYB1* genotype with apple fruit skin color phenotypes in a 'Golden Delicious' × 'Arlet' progeny.

Skin color phenotype	No. of seedlings	*MdMYB1-1* presence:absence	Association consistency
Red	71	60:11	85%
Non-red	26	3:23	88%
Total	97	63:34	86%

Table 2. Association of *MdMYB1* genotype with apple fruit skin color phenotypes in a 'Honeycrisp' × 'Cripps' Pink' progeny.

Skin color phenotype	No. of seedlings	*MdMYB1-1* presence:absence	Association consistency
Red	108	85:23	79%
Non-red	58	11:47	81%
Total	166	96:70	80%

What this study means to industry:
Apple fruit skin or peel color is an important contributor to nutrition, consumer preference, and market value. The current form of this marker has greater than 80% predictability and could be a useful tool for apple marker-assisted breeding, particularly where 'Golden Delicious' is a parent. Similar to ethylene gene markers, this genotype (banding pattern) data are also important for informative selection of parent combinations to produce desirable combinations and to design selection strategies.

3. Characterization of cultivar differences in alcohol acyltransferase (AAT) and 1-aminocyclopropane-1-carboxylate synthase (ACS) gene expression and volatile ester emission during apple fruit maturation and ripening.

(Collaborators: Jim Mattheis and Dave Rudell)

Alcohol acyltransferase (AAT) catalyzes the last step of volatile ester biosynthesis, and ethylene purportedly regulates AAT gene expression. In this study, expression patterns of apple AAT genes and ethylene biosynthesis genes of 1-aminocyclopropane-1-carboxylate synthase (ACS) were investigated in cultivars with relatively high ('Golden Delicious') or low ('Granny Smith') volatile ester production. All four AAT genes expressed stronger in 'Golden Delicious' than in 'Granny Smith'. MdAAT1 and MdAAT2 are the predominant genes expressed in fruit tissues. The expression levels of MdAAT1 and MdAAT2 increased as ripening progressed and were consistent with the total amount of esters detected between two cultivars. The transcript levels of MdAAT3 and MdAAT4 decreased at or after the onset of ripening. The expression of MdACS1 significantly increased at the onset of ripening while the expression of MdACS3 was detected throughout the harvest period. Postharvest 1-MCP exposure had little impact on expression of MdAAT1 and MdACS3 genes, but substantially suppressed the transcript level for MdACS1 in both cultivars and MdAAT2 in 'Golden Delicious'. The results indicated that: 1) differential expression of AAT genes may contribute to phenotypic variation of volatile ester biosynthesis;, 2) MdACS3 may play a role in induction of AAT gene expression in early fruit development asACS3 is expressed prior to ACS1; 3) climacteric expression of MdACS1 greatly enhanced the expression levels of MdAAT1 and MdAAT2 genes and the emission of aromatic volatile esters; 4)

postharvest 1-MCP treatment resulted in selective inhibition of gene expression for specific AAT and ACS family members.

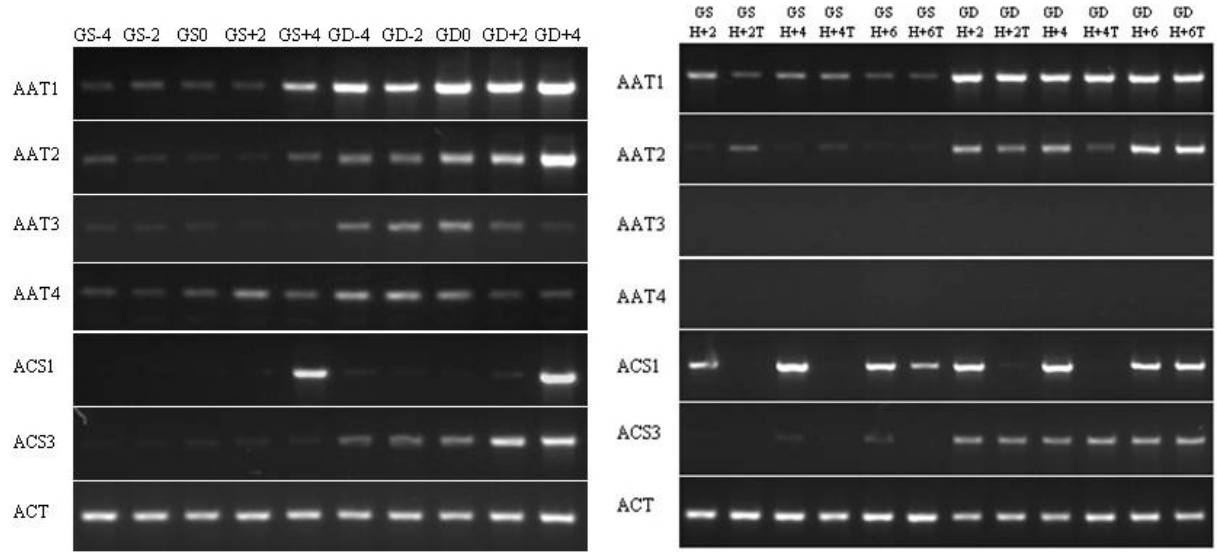

Figure 2. Expression patterns of four AAT genes and two ACS genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue at different ripening stages.

Left panel: Fruit peel tissues with two-week intervals beginning 128 (GD-4) or 149 (GS-4) days after full bloom (DAFB) for 'Golden Delicious' (GD), 'Granny Smith' (GS), respectively. Top axis label indicates cultivar and weeks prior to (designate "-"), at (0), or after (+) physiological maturity was attained. **Right panel:** Expression patterns of four AAT genes in 'Granny Smith' and 'Golden Delicious' apple peel tissue during ripening after harvest. Fruit were harvested 156 (GD0) or 177 (GS0) days after full bloom (DAFB) and held up to 6 weeks at 20 ^{o}C. Top axis label indicates cultivar, weeks after harvest (H+2, H+4 and H+6), and "T" indicates treatment at harvest with 1-MCP.

What this study means to industry?
Production of volatile esters varies significantly among apple cultivars, while the genetic controls underlying these differences have not been elucidated.
Cultivar differences in AAT gene expression intensity, particularly for AAT1 and AAT2, are in principal correlated with the levels of total detected emission of volatile esters. This study represents the first step in assigning function of these genes in aroma production. Further tests of the association between AAT gene expression and phenotypes in wider spectrum of cultivars or germplasm may enable development of a functional DNA marker for practical use in breeding program.

4. Transcriptomic analysis of cultivar-specific apple fruit ripening and texture attributes
(Collaborators: Dorrie Main, Jim Mattheis, Eric Curry)

Molecular events regulating cultivar-specific apple fruit ripening and sensory quality are largely unknown. Such knowledge is essential for genomic-assisted apple breeding and postharvest quality management. In this study, transcriptomic analysis, scanning electronic microscopic examination and systematic physiological characterization were performed on two apple cultivars, 'Pink Lady' (PL) and 'Honeycrisp' (HC), which have distinct ripening behavior and texture attributes. Substantial differences of crispness and firmness in fruit cortex were observed. SEM images of fruit cortex tissues prepared from

fruits with similar developmental stage suggest that the cell wall thickness may contribute to the observed firmness and crispness phenotype. A high-density long-oligo apple microarray consisting of duplex 190,135 cross-hybridization-free 50-70-mer isothermal probes, and representing 23,997 unigenes was manufactured on a Nimblegen array platform. The developmental stage- and cultivar-specific expression profiling analysis and QPCR validation indicated that genes in several functional groups express differentially between cultivars and ripening stages.

A. Define ripening stage in both cultivars for selecting fruit cortex tissues transcriptome analysis.

For each cultivar, tissues from three time points representing commercial maturity (0), 4 weeks before (-4) or 2 weeks before (-2) commercial maturity were used for transcriptome analysis (Table 3). Four biological repeats of fruit cortex tissues along with fruit maturity data were collected for each time point.

Table 3. Physiological characterization of fruit maturity and texture attributes for 'Honeycrisp' and 'Pink Lady' apple cultivars.

'Honeycrisp'						
Weeks before commercial harvest	-4	-3	-2	-1	0	+1
Sample date	Aug 5	Aug 12	Aug 19	Aug 26	Sep 2	Sep 9
IEC ($ul \cdot l^{-1}$)	0.11	0.01	<0.01	0.06	0.19	0.18
Firmness (N)	92.3	79.7	75.2	69.8	61.2	60.8
Starch pattern index (1-5)	1.0	1.0	1.3	1.9	3.8	4.5
Cn value	200	273	321	240	268	292
'Pink Lady'						
Weeks before commercial harvest	-4	-3	-2	-1	0	+1
Sample date	Oct 7	Oct 14	Oct 21	Oct 28	Nov 4	Nov 12
IEC ($ul \cdot l^{-1}$)	0.16	0.01	0.24	< 0.01	1.12	0.46
Firmness (N)	103.5	111.6	101.3	97.2	94.5	94.1

All values are means based on a weekly sample of 15 apples. Fruit firmness was evaluated using a Mohr Digitest. Crispness (Cn) is defined as high frequency tearing characteristics of fruit material. Rating of starch pattern index was based on iodine staining and scored using Cornell composite standards. Internal ethylene concentration (IEC) was determined by GC using established methods.

B. Scanning electron microscopy (SEM) of apple fruit cortex cellular and cell wall features of both Pink Lady and Honeycrisp.

Microscopic cellular features in fruit cortex such as cell size, cell number, cell wall properties and intercellular space between adjacent cells may contribute to cultivar-specific fruit texture attributes. As shown in figure above, SEM images revealed no obvious differences on cell size and cell number per unit area from the cortex tissues between cultivars with equivalent maturity (top strip, 400x magnifications). With 2,000x magnification, the images indicated different cell inner face appearance and textural feature between two cultivars, i.e. a fine and smooth inner surface of cell wall from HC and rough and fortified texture of cell walls of PL cortex cells (middle strip in figure x). At 20,000x magnification, the

differences on thickness cell walls were readily visible; showing a decreasing trend of cell wall thickness and somewhat increasing thickness in PL were also observed (the bottom strip in figure x). (fruit cortex tissues labeled with stars were used for transcriptomic study).

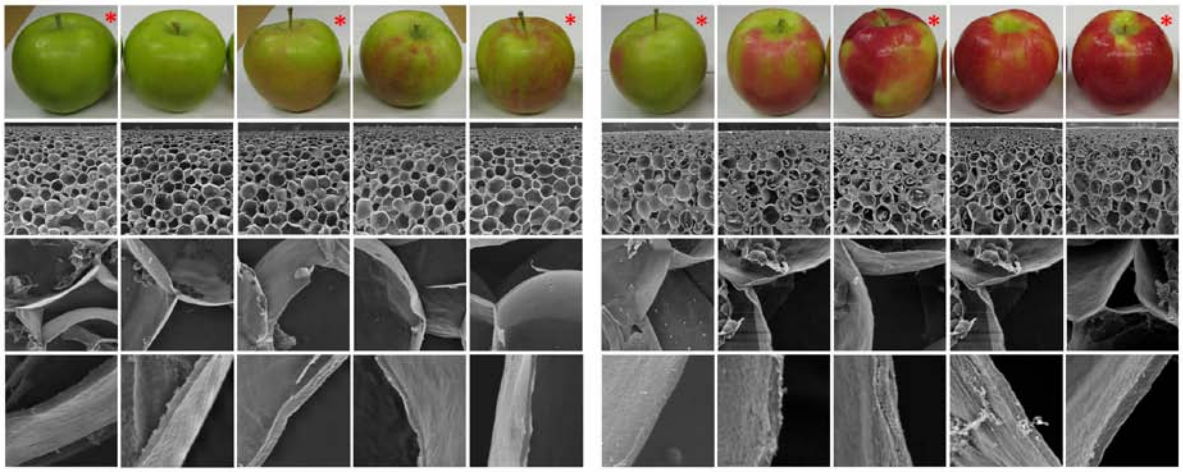

Figure 3. Representing fruit image and SEM images of fruit cortex cells with the maturity, from left to right at -4, -3, -2, -1 and 0 week.

Left panel: 'Honeycrisp'. Right panel: 'Pink Lady'. For both panels, top strip: fruit images represent the developmental stages expressed as week before commercial maturity. Second strip: 400 x magnification showing cellular features of cortex tissues. Third strip: 2000 x magnification showing cell wall. Bottom panel: 20,000 x showing close-up of cell wall.

C. Design of Nimblegen apple long oligo microarray and array hybridization.

A long oligo microarray was designed for apple based on the Malus unigene V4 sequences (Genome Database for Rosaceae, Jung *et al*., 2008). 260,581 Malus EST sequences were downloaded from NCBI dbEST (Benson *et al*., 2007), filtered for contamination and low quality sequence and assembled into 23,284 contigs and 53,200 singletons using CAP3 (Huang and Madan, 1999) with an overlap percentage parameter of 90 (-p 90). The unigenes, comprising of the combined contigs and singlets, were computationally annotated for putative function by pairwise comparison against the Arabidopsis thaliana protein database (www.arabidopsis.org), and the Uniprot Swiss-Prot and TrEMBL databases (Wu *et al.*, 2006; Mulder *et al.*, 2007) using the BLASTX algorithm. Only matches with an E-value of less than 1.0 e-6 were recorded. Based on the similarity search results, 55,960 (73%) of the Malus unigene sequences had significant matches with proteins from these databases. Array hybridization, data acquisition and normalization were done in Nimblegen Lab (Iceland).

D. Transcriptomic changes in apple cortex during ripening

By ANOVA analyses using a non-adaptive false discovery rate (FDR) of 0.01 and a cutoff value of 2-fold change of detected signal strength between any one of the two adjacent time points, a total of 1796 differentially expressed unigenes from HC and 1213 from PL were identified, representing 5% and 7.5% of all unigene deposited on the array, respectively. Unigenes with "unknown function", defined as those sequences with no match against a protein in TrEMBL, Swiss-Prot or Arabidopsis protein database, consisted of an average 33.2% of all identified differentially expressed unigenes. In each cultivar,

unigenes showing down-regulated expression patterns greatly outnumbered the ones showing up-regulated expression patterns. A slight higher percentage of unigenes as "up-regulated" was identified from late-ripening cultivar PL (34.7%) than that from early-ripening cultivar PL (31.3%). Based on their functional annotations, three functional groups were apparently regulated during the late stage of fruit ripeing.

Hormonal metabolism and response Transcriptomic changes related to plant hormone biosyntheses and responses are a major characteristic during late ripening stages of apple fruit. Over half of the unigenes (40 out of total 76) in this category are directly related to auxin and ethylene metabolism and response. Even more unigenes if specific transcription factors are included, such transcriptomic change suggests the central roles for these two hormones. Based on the number of unigenes with up- or down-regulated expression patterns, it seems that the roles of auxin were attenuating, while the effects of ethylene were strengthening during the period of 4 weeks before commercial maturity. Unigenes implicated in other hormone functions including gibberellin, brassinosteroid and jasmonate were also identified. Asummarized list is presented in Table x. **Ethylene:** ACS3 showed steadily increased expression patterns in both cultivars as on-tree development progressed, but substantially higher transcript levels of ACS3 were observed in HC. The expression level of ACO1 increased dramatically in HC, but just moderately in PL. Coincident with the low abundance of ACS3 transcript in PL, several unigenes encoding EIN3-binding F-box protein were up-regulated only in PL. Differential gene expression patterns were also observed for unigenes related to auxin metabolism and response between these two cultivars. **Auxin:** Five unigenes annotated as "auxin efflux carrier component" were specifically down-regulated in PL; in contrast, a unigene with similar annotation was only up-regulated in HC. Several unigenes related to the regulation of auxin homeostasis were also differentially regulated during fruit ripening. A unigene encoding indoleacetamide hydrolase functioning in auxin biosynthesis was down-regulated only in PL. However, a unigene annotated as a probable indole-3-acetic acid-amido synthetase GH3.5 is down-regulated only in HC. Conversely, a unigene with similar annotation was up-regulated in PL. More than a dozen "AUX/IAA proteins", the auxin response repressor, were also identified. The data seem to suggest that IAA is more readily available in the fruit cortex tissues of HC, and a positive correlation exists between auxin availability and active ACS3 and ACO1 expression. **Gibberellin**: four unigene encoding "gibberellin 3 (or 2)-beta-dioxygenase" for GA biosynthesis, four unigene for "DELLA proteins" (nuclear repressors of GA-responses) were identified as differentially expressed genes. **Jasmonate:** Four Jasmonate O-methyltransferase encoding unigenes were selectively up-regulated in both cultivars, while in contrast all ten unigenes annotated as "BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor" were selectively down-regulated in both cultivars. In many cases the number of identified unigenes can be reduced pending sequence analysis, as often multiple unigenes are associated with the same protein identity; therefore they may be different alleles for the same gene.

Transcription factors (TFs) TFs are proteins with sequence-specific DNA binding ability which may activate or repress transcription of sets of genes in response to endogenous and exogenous stimuli (Riechmann et al., 2000). Based on available EST data, an estimated 1025 unigenes encode 62 transcription factor (TF) families, and more than 90% of these TFs have orthologs in other species (Guo et al, 2008). A extensive number of TF-encoding unigenes were differentially expressed in both cultivars, i.e. 82 of them from PL and 142 of them from HC, indicating a dramatic transcriptional regulation at late fruit ripening stages. Consistent with the strong presence of gene activity related to auxin and ethylene biosyntheses and responses, almost a quarter of TFs encoding unigenes belong to those specifically responding to auxin and ethylene. The majority of unigenes encoding TFs were down-regulated as fruit ripening progressed, except ethylene responsive TFs. Transcriptional regulation controls many biological processes, which is achieved primarily through the actions of transcription factors (TFs). A table below summarizes the identity of the classification of identified TFs.

Table 4. No. of differentially expressed unigenes encoding proteins in various transcription factor (TF) families

TF family	Up-regulated in PL	Down regulated in PL	Up-regulated in HC	Down-regulated in HC
Ethylene-responsive family	11	6	4	8
Auxin-responsive TF family	1	10	3	13
Br responsive family	0	3	0	2
bHLH family	1	5	1	3
bZIP	0	3	1	1
Homeobox-leucine zipper	1	7	4	7
Myb protein family	7	3	4	10
NAC domain TF family	2	2	2	10
RING-H2 finger	1	1	3	2
SBP-like	0	2	0	3
Zinc finger family	0	2	1	19
Dof zinc finger protein	0	2	0	10
CONSTANS-like protein	0	1	0	2
WRKY transcription factor	0	0	4	6
MADS-domain protein	1	0	0	0
Other	3	3	5	6

Cell wall metabolisms Unigenes belonging to several gene families encoding proteins that modify specific cell wall components were identified in cortex tissues. While most of these genes are similarly regulated between two cultivars, a few unigenes showed strong cultivar-specific expression patterns. The most noticeable groups of unigenes were those involved in hemi-cellulose degradation. Four xyloglucan endotransglycosylase (XET) encoding unigenes were identified with differentially expressed patterns. All three XET genes identified from HC exhibited increased expression patterns, while only one showed increased pattern and two other exhibited decreasing expression patterns in PL. All of the identified four "probable pectate lyase" encoding unigenes were down-regulated in PL, but only 2 of them exhibited down-regulated expression patterns in HC. A down-regulated "pectinesterase inhibitor" encoding unigene was detected only in PL, but an up-regulated "putative xyloglucanase inhibitor" encoding unigene was only detected in HC. Of three unigenes for "beta-galactosidase", one from each cultivar showed down-regulated expression, while an extra unigene was up-regulated only in PL. A unigene encoding for a 6(G)-fructosyltransferase was significantly up-regulated only in the first two-week period of fruit ripening in HC.

What this study means to industry

Genetic controls and molecular mechanisms of apple fruit ripening and fruit quality are the most critical component in tree fruit genomics study, yet very limited knowledge current exists. These results represent an initial step to identify genes responsible for unique fruit ripening behavior and fruit quality traits. Specifically, two aspects are expected to contribute to the sustainability and profitability of fruit industry, i.e. developing tools for genomics-assisted breeding and innovative fruit quality management.

Four peer reviewed publications related to this proposal:

Zhu Y and Barritt BH. 2008. Md-ACS1 and Md-ACO1 genotyping of apple (*Malus x domestica* Borkh.) breeding parents and suitability for marker-assisted selection. Tree Genetics and Genomes 4: 555-562.

Zhu Y, Rudell, DR, Mattheis JP. 2008. Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1-carboxylate synthase gene expression and volatile ester emission during apple fruit maturation and ripening. Postharv Bio Technol 49: 330-339.
Zhu Y, Evans K and Peace C. 2010. Utility testing of an apple skin color MdMYB1 marker in two progenies. Molecular Breeding (submitted).
Zhu Y, Zheng P, Varanasi V, Main D, Curry E and Mattheis JP. 2010. Transcriptomic analysis of cultivar-specific apple fruit ripening and texture attributes. BMC Plant Biology (in preparation)

EXECUTIVE SUMMARY

Project Title: Functional genomics and marker development for apple sensory qualities

The essence of genetic studies is to establish gene-traits associations. The knowledge from such studies provides the basis for **genomics-assisted breeding** and **genomics-based quality management**. Breeding for locally adapted apple cultivars requires a long-term commitment due to the crop's perennial nature, long-juvenile phase, and high heterozygosity of the genome and poor predictability of fruiting performance. Implementation of a marker-assisted seedling selection strategy, such as using gene-specific functional molecular markers, is especially beneficial for perennial tree fruit breeding. By implementing genotype-directed selection, less desirable seedlings (such as those with non-red skin fruit) can be culled at an early stage prior to actual fruiting, therefore minimizing future orchard planting costs, tree maintenance, and performance evaluation. Breeding efficiency can also be increased by using DNA information to design crosses that lead to a greater proportion of desirable seedlings, such as all seedlings having fruit with red skin, or at least a predicted proportion of seedling outcomes.

Testing and seeking implementation of three reported apple gene-specific DNA markers, two for **ethylene biosynthesis** potential and one for **apple skin color,** in an on-going Washington State Apple Breeding Program has never been reported. These studies provide guidelines for potential utilization in apple breeding. As current understanding of tree fruit genetics is very limited, more research is required to better understand the molecular mechanisms regulating fruit quality traits. In addition to testing currently available markers, this research also included genetic analysis of apple fruit related to **firmness, crispness** and **aromatic volatile ester production**. Using gene expression profiles, candidate genes were compiled for further examination. The results from this study set the foundation for further functional analysis to associate specific genes with apple firmness, crispness and volatile ester production, with the ultimate goal to develop functional molecular markers for these traits.

The achievements from this project were largely due to the close collaboration among researchers; a total of six labs with different research expertise were cooperatively contributing to the progress including bioinformatics from **Dr. Main**'s lab, physiological characterization in **Dr. Mattheis**' lab, germplasm availability from on-going breeding program managed by **Dr. Barritt** and **Dr. Evans**, scanning electronic microscopic study in **Dr. Curry**'s lab, and high through-put genotyping facility in **Dr. Peace**'s lab. While our study has been closely aligned with the needs of the tree fruit industry, these research activities also generate essential data for scientific advancement in apple genomics as evident by several peer-reviewed publications.