FINAL PROJECT REPORT

Project Title: Developing flavor gene markers for the WA tree fruit industry

PI:	Cameron Peace	**Co-PI(2)**:	Jim Olmstead
Organization:	Washington State University	**Organization**:	WSU Prosser
Telephone:	509-335-6899	**Telephone**:	509-786-9249
Email:	cpeace@wsu.edu	**Email**:	jwolmstead@wsu.edu
Address:	Dept Hort. Landsc. Arch.	**Address**:	IAREC
Address 2:	39 Johnson Hall	**Address 2**:	24106 N. Bunn Rd
City:	Pullman	**City**:	Prosser
State/Zip:	WA 99163	**State/Zip**:	WA 99350

Co-PI(3):	Bruce Barritt / Kate Evans	**Co-PI(4)**:	Rod Drew
Organization:	WSU Wenatchee	**Organization**:	Griffith University
Telephone:	509-663-8181 ext 233	**Telephone**:	+61-7-3875-7292
Email:	kate_evans@wsu.edu	**Email**:	r.drew@griffith.edu.au
Address:	TFREC	**Address**:	Kessels Rd
Address 2:	1100 N. Western Ave	**Address 2**:	Nathan
City:	Wenatchee	**City**:	Brisbane
State/Zip:	WA 99801	**State/Zip**:	Qld 4111, Australia

Cooperators: Amy Iezzoni, Wayne Loescher, Randy Beaudry, Steve van Nocker (Dept Horticulture, Michigan State University), Dorrie Main, John Fellman, Amit Dhingra (Dept Horticulture & LA, WSU Pullman), Nnadozie Oraguzie (Dept Horticulture & LA, WSU Prosser), Yanmin Zhu (USDA-ARS, Wenatchee), Carolyn Ross (Dept Food Science & Human Nutrition, WSU Pullman), Eric van de Weg, Marco Bink (Plant Research International, Netherlands) , Eben Ogundiwin (UC Davis, California), David Chagne and Susan Gardiner (Plant & Food Research, New Zealand), Jim McFerson (WTFRC, Wenatchee), Fred Bliss (Davis, California)

Other Funding Sources

	As PIs	As co-PIs only
Total completed	$356,623	
Total current	$851,710	$77,616
Total to begin soon		$11,500,000

Completed

Agency Name: WTFRC Apple Review
Amount awarded: $158,422 in 2008
Notes: "Apple scion breeding". Barritt PI, Peace co-PI. Synergistic project and beneficiary of flavor gene advances for apple.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $80,893 in 2008
Notes: "Breeding and genetics program for Pacific Northwest cherries". Olmstead PI. Synergistic project and beneficiary of flavor gene advances for sweet cherry.

Agency Name: WTFRC Apple Review
Amount awarded: $40,575 in 2007

Notes: "Adapting available genomics tools to enhance WA apple breeding". Peace. Synergistic project on marker-assisted breeding application for the WSU apple breeding program.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $67,900 in 2008
Notes: "Adapting available genomics tools to enhance Pacific Northwest sweet cherry breeding". Peace, Olmstead. Synergistic project on marker-assisted breeding infrastructure for the WSU sweet cherry breeding program.

Agency Name: Prunus CGC
Amount awarded: $8,833 in 2008
Notes: "Characterization of principle flavor components in the *Cerasus* subgenus members of the *Prunus* Germplasm Collection". Olmstead PI. Synergistic project – phenotypic data collection.

Current

Agency Name: WTFRC Apple Review
Amount requested: $169,210 in 2009
Notes: "Apple Scion Breeding". Evans PI, Peace co-PI. Synergistic project and beneficiary of flavor gene advances for apple.

Agency Name: WTFRC Apple Review
Amount awarded: $87,500 in 2009
Notes: "Genetic marker assistance for the Washington apple breeding program". Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding application for the WSU apple breeding program.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $45,000 in 2009
Notes: "Establishing the Marker-Assisted Breeding Pipeline for sweet cherry". Peace PI and Olmstead co-PI. Synergistic project on marker-assisted breeding infrastructure for the WSU sweet cherry breeding program.

Agency Name: WTFRC Apple Review
Amount awarded: $77,616 in 2009
Notes: "Developing an online toolbox for tree fruit breeding". Peace, Olmstead, and Evans co-PIs (PI: D. Main). Synergistic project – bioinformatics support for WSU apple and sweet cherry breeding programs.

Agency Name: WTFRC Technology Review
Amount awarded: $50,000 in 2009
Notes: "ABI 3730 DNA Analyzer to augment tree fruit breeding and research" Peace PI. Matched with $50,000 from Washington Wheat Commission (separate award, PI: D. See) to obtain refurbished ABI 3730 DNA Analyzer for high-throughput genotyping of tree fruit and cereals, based in Pullman.

Agency Name: WSU Agricultural Research Center
Amount awarded: $100,000 in 2009
Notes: Additional support to Dr. Peace and the "Pacific Northwest Tree Fruit Genotyping Laboratory" for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping.

Agency Name: USDA-CSREES, National Research Initiative

Amount awarded: $400,000 in 2008-2010
Notes: "Functional gene markers for Rosaceae tree fruit texture". Peace PI. Synergistic project – fruit texture genetic control in apple with emphasis on ethylene.

To begin soon

Agency Name: National Science Foundation
Amount awarded: $2,000,000 for July 2009 – June 2013
Notes: "Genome Database for Rosaceae". Peace and Olmstead co-PIs (PI: D. Main). Synergistic project to develop broad bioinformatics support for Rosaceae crops.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $2,000,000 for September 2009 – August 2013
Notes: "Tree Fruit GDR: Translating genomics to fruit tree agriculture". Peace and Evans co-PIs (PI: D. Main). Synergistic project for practical application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7,500,000 for September 2009 – August 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". Peace and Evans co-PIs (PI: A. Iezzoni). Broad umbrella project on genetic marker development and application that effectively supersedes the current flavor gene project.

Total Project Funding: $27,035

Budget History:

Item	Year 1: Jul07-Jun08	Year 2: Jul08-Jun09	Year 3:
Salaries	4552		
Benefits	1548		
Wages	6895		
Benefits	2040		
Equipment			
Supplies	12000		
Travel			
Miscellaneous			
Total	27035	0	

RECAP ORIGINAL OBJECTIVES

This project seeks to **develop a generic system that identifies genes controlling traits of importance to the Washington tree fruit industry**, and to **implement this system using the example of fruit flavor**. Understanding the key control points for traits of interest is very valuable for improving crop production. This knowledge can then be put to practical use, such as in marker-assisted breeding, controlled sport induction, or chemical genomics. The project uses the candidate gene approach and capitalizes on expanding genomics databases and a large international network of tree fruit genomics, genetics, and breeding researchers. Specific objectives are to 1) develop DNA tests of flavor useful for Washington tree fruit breeding programs, 2) establish a temperate-tropical fruit genomics channel through linkages between the Rosaceae and papaya genomes, and 3) identify tropical fruit flavor genes having potential value for the Washington tree fruit industry.

While originally planned for three years, after two years of significant progress in this and companion projects, advances international in genomics, and recent success in new federal grant funding, we believe that the next steps to achieving the project's major goal are best made within the new federal projects of "RosBREED: Enabling marker-assisted breeding in Rosaceae", "Genome Database for Rosaceae", and "Tree Fruit GDR: Translating genomics to fruit tree agriculture", and the current WTFRC-funded projects on genetic marker assistance for WSU's apple and sweet cherry breeding programs.

SIGNIFICANT FINDINGS

- Our multi-pronged approach to channel genomics knowledge of fruit flavor (particularly sweetness, acidity, and aroma) into the WSU tree fruit breeding programs has successfully established major components of a system for genetic marker identification, and has generated several DNA tests with promise for the Pacific Northwest apple and sweet cherry industries.

- A "Master List" of 100 candidate genes with potential contribution to the formation of sweetness, acidity, and volatile aroma compounds were assembled. These genes provide valuable focal points for mining the whole genome sequences of apple and peach when publicly released later in 2009.

- Gene expression analyses by Dr. Randy Beaudry at MSU for volatile aroma candidate genes identified three classes of genes of particular interest, incorporated into the Master List.

- Preliminary whole genome sequencing of sweet cherry by Dr. Amit Dhingra was functionally connected with marker development within this flavor gene project.

- A "Flavor Gene Map" was created for displaying the many genomic regions relevant to flavor, to guide genetic marker development.

- Genomic regions in sweet cherry correlated with sweetness, acidity, individual sugars, and malic acid were placed on the Flavor Gene Map with data from a companion project

- Several promising candidate genes that appear to underlie flavor QTLs (quantitative trait loci, which are genomic regions correlated with trait performance without the controlling gene necessarily known) were identified, and are undergoing a process of validation and utility testing within the apple and sweet cherry Marker-Assisted Breeding (MAB) Pipelines.

- This project has served as a focal point for supporting and collating flavor performance data on apple and sweet cherry germplasm. Insights into sugar profiles of cherry were made by comparing data sets from two companion projects. Novel sugar profiles and aroma profiles were identified in cherry and apple germplasm for exploitation in WSU breeding programs – particularly as we implement genetic markers for those traits.

- Phenotypic data on apple flavor attributes were obtained by Dr. Beaudry for 184 lines (42 species and two hybrid lines) from the Geneva *Malus* core collection. Measurements included internal ethylene concentration, SSC, "taste" (tart, astringent, sweet, nutty, floral, fruity, bland, acid, lemon, alcohol, spicy, anise, and other unusual notes), and volatile compounds (volatile esters, aldehydes, alcohols, and terpenoids).

- We were unsuccessful in 2007 and 2008 to receive Australian funding to support this project's specific objectives of establishing and implementing a temperate-tropical fruit genomics channel. Planned activities on this area for the final years of the project were therefore not conducted.

- Major federal funding has been obtained for two bioinformatics projects and one MAB Pipeline project, to begin in September 2009. Proposed Rosaceae community-wide work of those projects overlaps with and supersedes the originally planned system of this present flavor gene project.

RESULTS & DISCUSSION

Flavor attributes are important to the WA tree fruit industry and are high priority targets in the WSU apple and sweet cherry breeding programs. We have chosen and are testing genes that are likely suspects influencing in fruit flavor for apple and sweet cherry. We are also collecting and collating flavor-related performance data for experimental, breeding, and collection germplasm. Finally, Pedigree-Based Analysis combines the genotypic and phenotypic data to describe the effect of specific gene variants and their distribution in germplasm. With this system, we are transforming as many genes as possible into genetic screening tools for breeding, via the marker-assisted breeding approach. With marker-assisted seedling selection, the infrastructure for which we are developing in other projects, we can improve the efficiency of breeding operations by reducing the proportion of seedlings planted in the field with poor genetic value. Economic analyses within concurrent projects are providing bottom line figures that indicate substantial cost savings by implementing even one genetic marker that tags an important trait.

The Master List of flavor genes
Candidate genes with potential to contribute to the formation of sweetness, acidity, and volatile aroma compounds were assembled based on literature and advice from collaborators. This "Master List" contains (more than) 100 distinct gene sequences representing 51 types of genes (provided as Appendix 1 in the First Year Continuing Report, August 2008, and available on request).

Gene expression analyses by Dr. Randy Beaudry at MSU for volatile aroma candidate genes identified three classes of genes of particular interest, incorporated into the Master List. Several putative genes of branched-chain aminotransferase (BCAT), pyruvate decarboxylase (PDC), and 2-isopropylmalate synthase (IPMS) were found to have expression patterns that increase concurrently with branched-chain ester production (details provided in the August 2008 report).

Sweet cherry ('Stella') whole genome sequence data obtained by Dr. Amit Dhingra at WSU were screened to identify cherry versions of Master List flavor genes for which no cherry DNA sequences were previously known. These cherry DNA sequences will facilitate the development of efficient flavor gene tests for cherry, because differences among genomes can cause some tests developed on apple and peach sequences to fail when attempted for cherry. Previously, only 1-2% of the DNA sequences for flavor genes in Rosaceae that we could obtain from public databases were from sweet cherry.

Master List flavor gene annotation (characterization) needs have provided a useful test case for the Genome Database for Rosaceae program's efforts in developing bioinformatics tools for tree fruit breeding – the focus of the current WTFRC-funded project "Developing an online toolbox for tree fruit breeding" and the upcoming SCRI-funded project "Tree Fruit GDR: Translating genomics to fruit tree agriculture" directed by Dr. Dorrie Main at WSU. To date, 37 of the Master List flavor genes have been identified in both apple and peach (list attached), the combined sequences have been assembled using different assembly programs, and the best consensus sequences have been annotated for coding region using the gene prediction program fgenesh and the protein domain program Interproscan. As part of the newly funded GDR and RosBREED (see below) programs we will align these sequences against the peach and apple whole genome sequences to generate more detailed and higher quality comparative annotations which will then be used to identify optimal primer sequences using PRIMER3, for use in screening on specific breeding germplasm.

"RosBREED" is a U.S. Rosaceae community-wide project that mobilizes international genomics, statistical, germplasm, breeding, and phenotyping resources to establish and implement a Marker-Assisted Breeding Pipeline for Rosaceae crops. More than a thousand fruit quality genes will be chosen for apple, peach, strawberry, and cherry, their sequences and variation thoroughly described, and their genomic locations pinpointed. Subsequently, fruit quality" genome scans" will be developed and screened on hundreds of cultivars, selections, and seedlings representing the crops and core participating breeding programs (which include WSU's apple and sweet cherry). Collation of existing performance data plus new standardized data collected for three additional seasons will be combined with genome scans with Pedigree-Based Analysis to identify and characterize fruit quality genetic variation. As the flavor genes on the Master List will be included in the genome scans, RosBREED's planned activities exceed those intended in the present flavor gene project. Thus it is most efficient to allow further flavor gene marker development to be conducted within RosBREED.

The Flavor Gene Map
Major flavor trait-influencing regions and flavor candidate genes in the *Malus* (apple) and *Prunus* (stone fruit) genomes were combined into a single visual database, the Flavor Gene Map. This Map currently has 53 flavor candidate genes and 57 QTLs for sweetness, acidity, volatile aroma, and astringency (Figure 1). These regions were determined by:

a)   Surveying QTL literature on analyses in apple, peach, and cherry (Maliepaard et al. 1998; Wang et al. 2000; Etienne et al. 2002; Liebhard et al. 2003; Kenis et al. 2008; Dunemann et al. 2009). In recent scientific conferences, researchers at Plant & Food Research in New Zealand reported the location of a major locus (*2MBAc*) on chromosome 2 of apple controlling levels of the major volatiles contributing to typical apple aroma.

b)   Obtaining unpublished data generously provided by colleagues involved in genetic mapping of flavor candidate genes. At UC Davis, Dr. Eben Ogundiwin has recently placed 12 flavor candidate genes on the *Prunus* genome within a study that is developing a "*Prunus* fruit quality gene map" (Ogundiwin, Peace, et al., manuscript submitted). At Plant & Food Research, Dr. David Chagne and students (Mukarram Mohammed and Aurélie Dimouro) mapped 13 flavor

candidate genes from a list compiled by their flavor genomics specialists, Drs. Edwige Souleyre, Richard Newcomb, Robert Schaffer, and Ross Atkinson. That work was performed with the aim of identifying the gene controlling the *2MBAc* aroma locus, but thus far has not been successful. Nevertheless, the 13 candidate genes may be discovered to control other aroma loci, and so they are included on the Flavor Gene Map.

c) Considering also published gene locations of genes involved in the ethylene biosynthesis and perception pathway. Reports on transgenic apple fruit with the ACS and ACO genes knocked out (Dandekar et al. 2004; Schaffer et al. 2007) have shown that while sweetness and acidity development in fruit is apparently independent of ethylene, volatile aroma compounds – particularly volatile esters – are strongly affected by ethylene. The final enzymatic steps of volatile aroma compound synthesis are always affected by ethylene levels, and often the initial enzymatic step is also influenced (Schaffer et al. 2007). The genes for these volatile production enzymes are part of the Master List of flavor genes, while the ethylene genes are additional. Because low fruit ethylene production is a target for apple texture genetic improvement in the WSU apple breeding program, while low ethylene tends to result in lower aroma production, we seek flavor gene variants that enable pleasing aroma development in low ethylene genotypes.

d) Including cherry QTL data from the WTFRC-funded companion project "Adapting available genomics tools to enhance Pacific Northwest sweet cherry breeding" with Dr. Amy Iezzoni at MSU. Several QTLs were located for flavor components (SSC, absolute and relative sugar contents, total soluble solids, malic acid, and astringency) using the MSU experimental population of NYxEF (a cross between the wild cherry NY54 and heritage cultivar Emperor Francis).

Many cases were observed on the Flavor Gene Map where candidate genes for a flavor trait were at the same region as chromosomal regions influencing a flavor trait (i.e. QTLs) (Figure 1). As the map aligns kindred apple (*Malus*) chromosomes, and aligns ancestrally tied apple and cherry/peach (*Prunus*) chromosomes, QTLs for one crop were often in the same region as for other crops, and some of the gene-QTL co-locations were also across chromosomes, offering additional opportunities for gene function validation and utility assessment. Some of the opportunities are already being investigated further:

- At the bottom end of *Malus* chromosome 16 is located a major QTL for acidity, and a candidate gene for acidity showed up in the same place. This is also a region strongly influencing crispness and juiciness. Predictive tests for these traits based on the this chromosome 16 region are being developed within the apple MAB Pipeline (companion WTFRC-funded project "Genetic marker assistance for the Washington apple breeding program").
- The gene controlling the *2MBAc* locus on *Malus* chromosome 2 is not yet known, but we are tagging the region with nearby markers (with helpful genomic information provided by David Chagne at Plant & Food Research), and investigating its usefulness for predicting apple aroma presence/absence for parents and seedlings of the WSU apple breeding program.
- In an aligned region on Prunus chromosome 4 and Malus chromosomes 10 and 5 are major QTLs for sweetness, acidity, and volatiles, and candidate genes for volatiles. This region also holds major QTLs for texture. The synergistic NRI-funded project "Functional gene markers for tree fruit texture in Rosaceae" is examining such linkages among traits in detail by measuring texture and flavor performance of a large apple germplasm set and studying several genes and markers in this region. That project will consider how selection for specific texture gene variants here would affect flavor attributes – ultimately to allow efficient selection for the best combination of both.

Other opportunities abound, thanks to the consolidation of otherwise widely dispersed data that the Flavor Gene Map provides. The Map will continue to be updated with locations of flavor genes and QTLs as they are discovered and reported. Arising opportunities for developing useful predictive

DNA tests for flavor in Washington tree fruit will be exploited in the WTFRC-funded genetic marker assistance / MAB Pipeline implementation projects.
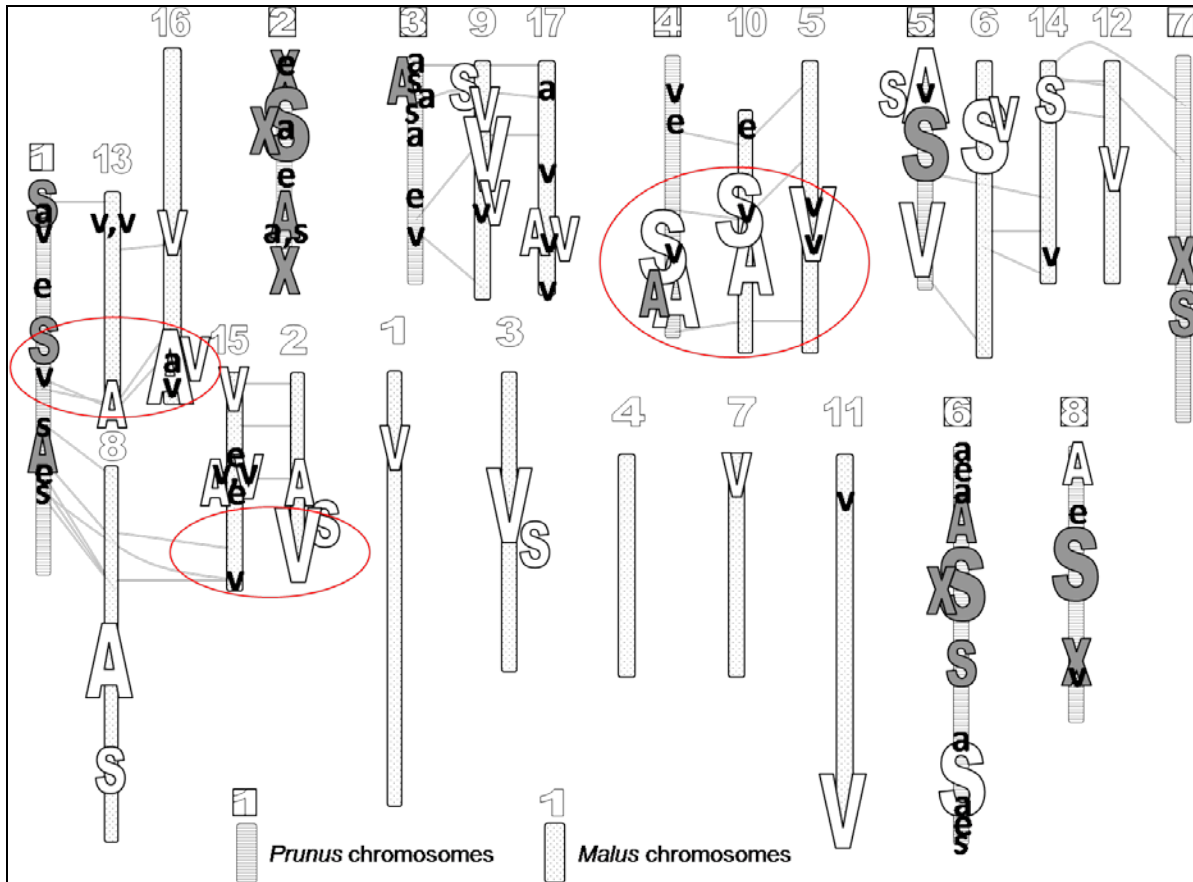

Figure 1: The **Flavor Gene Map** for apple and sweet cherry. S,s = sweetness, A,a = acidity, V,v = volatile aroma, x = astringency, e = ethylene. Chromosomal regions (QTLs – quantitative trait loci) influencing fruit flavor in the genomes of pome fruit (*Malus*) and stone fruit (*Prunus*) are indicated in block capital letters (larger letters = larger reported effect). Locations of candidate genes for flavor and ethylene synthesis/perception are shown in lower case letters. Horizontal lines show known connections between the related genomes. Three co-locations of candidate genes and QTLs targeted for further marker development are circled.

Flavor Phenotyping
This project has served as a focal point for supporting and collating flavor performance data on apple and sweet cherry germplasm. Two examples are described below.

Insights into sugar profiles of cherry were made by comparing data sets from two companion projects. The first data set was collected in collaboration with Dr. Dave Rudell at USDA-ARS Wenatchee, within the 2008 project "Characterization of principle flavor components in the *Cerasus* subgenus members of the *Prunus* Germplasm Collection". Approximately 80 accessions from the USDA cherry collection at Davis were measured for SSC, titratable acidity, and individual sugars and acids via gas chromatography-mass spectrometry (GC-MS). The second data set was for the MSU

population of NYxEF, described in the previous section. Sugar and acid contents and proportions were compared between these two data sets, and revealed opportunities for introducing different and desirable sugar profiles into sweet cherry by (marker-assisted) breeding. These opportunities are being exploited in the breeding program. Astringency was also examined in the NYxEF population, and from the resulting data it appears that marker-assisted breeding can readily incorporate breeding against high astringency – which will improve the efficiency of using wild sources as parents.

Phenotypic data on apple flavor attributes were obtained by Dr. Beaudry for 184 lines (42 species and two hybrid lines) from the Geneva *Malus* core collection. Measurements included internal ethylene concentration, SSC, "taste" (tart, astringent, sweet, nutty, floral, fruity, bland, acid, lemon, alcohol, spicy, anise, and other unusual notes), and volatile compounds (volatile esters, aldehydes, alcohols, and terpenoids). As for cherry, this data provides opportunities for introducing unique flavor profiles, particularly for aroma, into breeding germplasm.

References:
Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004). Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research 13:373-384

Dunemann F , Ulrich D, Boudichevskaia A, Grafe C, Weber WE (2009). QTL mapping of aroma compounds analysed by headspace solid-phase microextraction gas chromatography in the apple progeny 'Discovery' x 'Prima'. Molecular Breeding 23:501-521

Etienne C, Rothan C, Moing A, Plomion C, Bodenes C, Svanella-Dumas L, Cosson P, Pronier V, Monet R, Dirlewanger E (2002). Candidate genes and QTLs for sugar and organic acid content in peach [Prunus persica (L.) Batsch]. Theoretical and Applied Genetics 105: 145-159

Kenis K, Keulemans J, Davey MW (2008). Identification and stability of QTLs for fruit quality traits in apple. Tree Genetics and Genomes 4:647-661

Liebhard R, Koller B, Gianfranceschi, L, Gessler C (2003). Creating a saturated reference map for the apple (Malus x domestica Borkh.) genome. Theoretical and Applied Genetics 106:1497-1508

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs PM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998). Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. Theoretical and Applied Genetics 97: 60-73

Schaffer RJ, Friel EN, Souleyre EJF, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma J-H, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD (2007). A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiology 144:1899-1912

Wang D, Karle R, Iezzoni AF (2000). QTL analysis of flower and fruit traits in sour cherry. Theoretical and Applied Genetics 100:535–544

EXECUTIVE SUMMARY

This project seeks to develop a generic system that identifies genes controlling traits of importance to the Washington tree fruit industry, and to implement this system using the example of fruit flavor.

Our multi-pronged approach to channel genomics knowledge of fruit flavor (particularly sweetness, acidity, and aroma) into the WSU tree fruit breeding programs has successfully established major components of a system for genetic marker identification, and has generated several DNA tests with promise for the Pacific Northwest apple and sweet cherry industries. Identifying genes with variants that influence important flavor components of apple and cherry will allow us to better understand the genetic value of parents and advanced selections of the WSU apple and sweet cherry breeding programs. The knowledge will also provide a means to genetically manage these traits for crop improvement.

- The project involved extensive collaboration with experts in the U.S. and other countries, particularly in collating resources and knowledge on flavor genetics.

- A "Master List" of 100 candidate genes with potential contribution to the formation of sweetness, acidity, and volatile aroma compounds were assembled. These genes provide valuable focal points for mining the whole genome sequences of apple and peach.

- A "Flavor Gene Map" was created for displaying genomic regions relevant to flavor, to guide genetic marker development.

- Several promising candidate genes that appear to underlie flavor QTLs (quantitative trait loci, which are genomic regions correlated with trait performance without the controlling gene necessarily known) were identified, and are undergoing a process of validation and utility testing within the apple and sweet cherry Marker-Assisted Breeding (MAB) Pipelines.

- Opportunities for introducing unique flavor profiles into Washington tree fruit breeding were identified and are being exploited.

- Major federal funding has been obtained for two bioinformatics projects and one MAB Pipeline project, to begin in September 2009. Proposed Rosaceae community-wide work of those projects overlaps with and supersedes the originally planned system of this present flavor gene project.

While originally planned for three years, after two years of significant progress in this and companion projects, advances international in genomics, and recent success in new federal grant funding, we believe that the next steps to achieving the project's major goal are best made within the new federal projects of "RosBREED: Enabling marker-assisted breeding in Rosaceae", "Genome Database for Rosaceae", and "Tree Fruit GDR: Translating genomics to fruit tree agriculture", and the current WTFRC-funded projects on genetic marker assistance for WSU's apple and sweet cherry breeding programs.