

FINAL PROJECT REPORT

Project Title: Genetic marker assistance for the Washington apple breeding program

PI: Cameron Peace
Organization: WSU Pullman
Telephone: 509-338-4786
Email: cpeace@wsu.edu
Address: Dept. of Hort & LA
Address 2: 39 Johnson Hall
City: Pullman
State/Zip: WA 99164

Co-PI(2): Kate Evans
Organization: WSU Wenatchee
Telephone: 509-663-8181 ext 245
Email: kate_evans@wsu.edu
Address: TFREC, Dept. of Hort & LA
Address 2: 1100 N. Western Ave
City: Wenatchee
State/Zip: WA 98801

Co-PI(3): Jim Olmstead
Organization: (no longer involved)
Telephone:
Email:
Address:
City:
State/Zip:

Co-PI(4): Jim Mattheis
Organization: USDA-ARS TFRL Wenatchee
Telephone: 509-664-2280 ext 249
Email: mattheis@tfrl.ars.usda.gov
Address: 1101 N. Western Ave
City: Wenatchee
State/Zip: WA 98801

Cooperators: Jim McFerson (WTFRC), Fred Bliss (Davis, California), Dorrie Main (WSU Pullman), Bruce Barritt (WSU, ret.), Yanmin Zhu and Dave Rudell (USDA-ARS Wenatchee), Deven See (USDA-ARS Pullman), Jim Luby (U Minnesota), Eric van de Weg and Marco Bink (Plant Research International), Fabrizio Costa, Sara Longhi, and Riccardo Velasco (IASMA), Sue Gardiner, Stuart Tustin, and David Chagne (HortResearch), Susan Brown (Cornell U), Phil Forsline and G. Fazio (USDA-ARS Geneva), Walter Guerra (Laimburg), Francois Laurens (INRA), Rozemarijn Dreesen, Wannes Keulemans, and Mark Davey (KULeuven), Amy Iezzoni (MSU), and others.

Other funding sources

Agency Name: USDA-CSREES National Research Initiative

Amount awarded: \$400,000 (2009-2011)

Notes: “Functional gene markers for Rosaceae tree fruit texture” PI: Peace. Co-PIs: Costa, van de Weg, Luby, McFerson, Gardiner, Hamblin, and Oraguzie. Closely coordinated with activities 2-4 of this WTFRC apple project.

Agency Name: WTFRC Apple Review

Amount requested: \$635,201 (2009-2011)

Notes: “Apple Scion Breeding” PI: Evans. Co-PIs: Peace, Ross, Zhu. The foundation program on which this reported WTFRC project builds.

Agency Name: WTFRC Apple Review

Amount awarded: \$77,616 (2009-2010)

Notes: “Developing an online toolbox for tree fruit breeding” PI: Main. Co-PIs: Evans, Oraguzie, Peace, Jung. Establishment of bioinformatics and databasing support to facilitate the translation of genomics information into application in WSU tree fruit breeding programs. Synergistic with activity 5 of this reported WTFRC project and SCRI project “Tree Fruit GDR” below.

Agency Name: USDA-CSREES Specialty Crops Research Initiative

Amount awarded: \$2,000,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)

Notes: “Tree Fruit GDR: Translating genomics into advances in horticulture”. PI: Main. Co-PIs include Evans and Peace. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES, Specialty Crops Research Initiative

Amount awarded: \$7,200,000 + equal matching from universities, industry (Sep 2009 – Aug 2013)

Notes: “RosBREED: Enabling marker-assisted breeding in Rosaceae”. PI: Iezzoni. Co-PIs include Peace, Main, and Evans. A synergistic project to establish sustainable marker-assisted breeding infrastructure for U.S. Rosaceae crops, using the Marker-Assisted Breeding Pipeline concept that involves Pedigree-Based Analysis.

Agency Name: WTFRC Cherry Review

Amount awarded: \$45,000 (2009)

Notes: “Establishing the Marker-Assisted Breeding Pipeline for sweet cherry” PI: Peace. Co-PIs: Olmstead, Iezzoni, and Oraguzie. Synergistic project for establishing equivalent marker-assisted breeding infrastructure for the PNW sweet cherry breeding program.

Agency Name: WTFRC Cherry Review

Amount awarded: \$88,600 (2010-2011)

Notes: “Marker-assisted breeding strategies for large fruit and self-fertility” PI: Peace. Co-PIs: Oraguzie, Iezzoni, and Whiting. Synergistic project for conducting marker-assisted breeding in the PNW sweet cherry breeding program.

Agency Name: WTFRC Cherry Review

Amount awarded: \$56,000 (2010-2011)

Notes: “Targeting the ethylene biosynthetic pathway to improve cherry quality” PI: Peace. Co-PIs: Wiersma, Oraguzie, and Whiting. Synergistic project investigating role of ethylene genes in cherry.

Agency Name: WTFRC Technology Review

Amount awarded: \$165,743 (2011-2012)

Notes: “Breeding in the 21st Century: Technology platform for fast breeding” PI: Dhingra. Co-PIs: Peace, Evans, Oraguzie. Synergistic project to assess methods of faster breeding.

Agency Name: WTFRC Technology Review

Amount awarded: \$9500 (2011)

Notes: “Ultra-low freezers for genomics, genetics, and breeding labs” PI: Peace. Co-PI: Dhingra. As the project title states.

Agency Name: WTFRC Technology Review, Washington Wheat Commission

Amount awarded: \$100,000 (2009)

Notes: WTFRC: “ABI 3730 DNA Analyzer to augment tree fruit breeding and research” PI: Peace. WWC: PI: Deven See. WTFRC and WWC funding matched to obtain a refurbished ABI 3730 DNA Analyzer for high-throughput genotyping of tree fruit and cereals, based at Pullman.

Agency Name: WSU Agricultural Research Center

Amount awarded: \$100,000 (2009)

Notes: Additional support to C. Peace for high-throughput DNA extraction and genotyping equipment, complementing the ABI 3730 and removing technical bottlenecks for routine tree fruit genotyping. Part of this support was used to leverage a further \$50,000 from the Washington Wheat Commission and \$8,000 from D. See’s USDA base funding to obtain a BioMek Laboratory Automation Station – a “robot” for high-throughput DNA extraction and genotyping sample preparation – operational since September 2009.

Total Project Funding: \$121,500 – exclusive of collaborative expenses

Budget History:

Item	Year 1: 2009	Year 2: 2010	
Salaries			
Benefits			
Wages	\$21,186	\$22,034	
Benefits	\$ 3,814	\$ 3,966	
Supplies	\$10,000	\$10,000	
Travel			
- In-state	\$ 2,000	\$ 2,000	
- Other	\$13,500		
Outreach	\$ 5,000	\$ 5,000	
Miscellaneous	\$13,000	\$10,000	
Collaborative Expenses			
- Stemilt RCA room rental	\$ 6,000		
- Crew labor	\$13,000		
Total	\$68,500	\$53,000	

Collaborative Expenses	Year 1: 2009	Year 2: 2010	
Stemilt RCA room rental	\$ 6,000		
Crew labor	\$13,000		
Total	\$19,000		

RECAP ORIGINAL OBJECTIVES

The overall goal was to provide comprehensive molecular genetics support for the WABP utilization of marker-assisted breeding. Specific objectives were to:

- 1) Establish a world-class long-term reference apple germplasm planting in Washington.
- 2) Obtain comprehensive fruit quality phenotypic data on representative industry and breeding stock of Washington and the nation.
- 3) Ensure Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators.
- 4) Enhance outreach efforts to demonstrate local impacts of genetic marker use for apple.
- 5) Create a high-throughput seedling genotyping database for the WABP.
- 6) Develop a DNA fingerprinting system for new cultivar releases from the WABP.
- 7) Continue to pipeline new markers for high priority traits into the WABP.

SIGNIFICANT FINDINGS

All seven Objectives were accomplished to some degree: 1) Completed, 2) Completed, 3) Completed, 4) Only half-completed, 5) Still in early stages, 6) Completed, 7) Completed, with massive infrastructure enhancement underway through the RosBREED project.

- Obj 1. The Apple Germplasm Library, a collection of up to 2700 trees serving as the germplasm base of the Washington apple breeding program and various genetic studies, was established, maintained, added to, presented, reported on, and studied.
- Obj 2. Fruit quality evaluations were successfully conducted by the WTFRC lab on hundreds of breeding and collection trees over three storage durations to dissect the genetic control of crispness, firmness, juiciness, sweetness, acidity, and storage disorders. Data collected is being used in an international USDA-funded National Research Initiative (NRI) project examining and exploiting the genetic control of apple texture (led by Peace).
- Obj 3. We established an international network of apple breeders, geneticists, collection curators, and fruit quality experts, and developed protocols for standardized fruit harvest and quality evaluation for internationally coordinated, powerful research studies.
- Obj 4. The success stories of use of the ethylene genes and other genetic marker applications in the Washington apple breeding program have been and continue to be described at various venues with diverse audiences. Efforts have integrated with the larger federally funded RosBREED project, with our local experiences providing the major MAB examples to date.
- Obj 5. Funding and expertise for developing a streamlined seedling genotyping database was increased with new collaborative partnerships (with Drs. Dorrie Main and Deven See).
- Obj 6. The foundation was developed for a DNA fingerprinting system that determines uniqueness and parentage of WSU selections to better characterize and protect selections and new cultivar releases. This system was used to deduce the parentage of 'WA 2' ('Splendour' × 'Gala'), confirm parentage of 'WA 5', and describe uniqueness of both. Genetic markers were also used to fix a nursery mix-up prior to Phase 2 planting, and to ensure relocated mother trees from TFREC to Columbia View were identical to their originals.

- Obj 7. Several promising genetic tests are at various stages in the MAB Pipeline for the highest priority breeding trait categories of texture, flavor, and appearance. Out the downstream end of the Pipeline, two ethylene gene markers for fruit storability are now routinely used support breeding parent and seedling selection decisions. Gene markers for flavor, appearance, and further texture components are poised for routine use from 2011.
- The existence of this industry-supported WTFRC-funded project provided a critical foundation for \$7.2 million to be awarded by the USDA for the RosBREED project, of which the Washington apple breeding program and its supporting molecular genetics program are major participants and beneficiaries – substantially multiplying the investment by Washington apple growers in new cultivar development.

RESULTS & DISCUSSION

Significance to industry and potential economic benefits

Germplasm is the basis and future – and innovative limit – of any breeding program. We now have a comprehensive locally accessible “library” of germplasm at the Sunrise Research Orchard, which among other familiarizing opportunities is showcased to industry each July since 2008 in annual Field Days. The Apple Germplasm Library includes diverse material harboring a wide spectrum of valuable traits that will be drawn from to produce WA’s future cultivars that sustain the industry. The Library also contains material that through genetic studies is aiding efforts to reduce the time it takes to incorporate novel traits into new cultivars. Growing this material locally finally ensures that local environmental adaptability and local industry suitability are entwined in this process.

Fruit quality evaluation data obtained will be mined in 2011 within our internationally networked USDA-funded National Research Initiative (NRI) project to refine genetic tests for storability, texture, and flavor optimization. These genetic tests will be applied to informing postharvest management through placement of cultivars into genetic potential performance groups, and to breeding decisions in crossing and seedling selection. Already, previous genetic knowledge on two genetic tests for fruit ethylene production has been put to practical use in breeding, and represents a world first among apple and related crops for routine marker-assisted seedling selection for fruit quality. However, interactions between these two tests, and interactions with other genetic components of texture and flavor, remain unclear. The NRI project began two years ago to address these issues and involves experts across the U.S., Europe, and New Zealand. These data and those from collaborating institutions will be used to determine the joint influence of key texture/storability genes, investigate their impact on flavor retention and storage disorders, and apply resulting knowledge in supporting breeding and industry management decisions – including refining the specific situations in which applications of the genetic tests are appropriate.

The most important feature of the ethylene genes, used now in parent selection, seedling selection, and to describe the genetic potential of new cultivars, is that they represent the first markers to be used routinely in this way. We do not intend that they be used always, and the main outcome of the NRI project will be to refine their application based on the extent to which they can predict fruit quality. Thanks to the path forged by the trail-blazing ethylene gene markers, more markers for further traits are now coming into use.

Data management tools completed (calculator of cultivar parentage and uniqueness) and being developed (database for high-throughput seedling genotyping) link in with a wider range of computer-based tools, databases, and interfaces being established: Dr. Dorrie Main’s “Breeders Toolbox”, RosBREED’s Breeding Information Management System, Dr. Main’s Tree Fruit Genome

Database Resources, and our seedling selection efficiency tool that is also being enhanced in RosBREED. Breeding and genomics are huge data generators, yet previously existing management tools were insufficient and inefficient to extract the full value from these data so that breeding decisions can be optimally informed. Our efforts in this area, and those of critical collaborators such as Dorrie Main, place the WABP atop the wave of data rather than awash in the ocean. Specifically from this project, we can now accurately confirm parentage of breeding selections, or deduce it where necessary. Parentage is one of the pieces of information that growers use when considering new cultivar adoption, yet this information is not always known from breeding records and rarely confirmed. DNA fingerprinting to establish cultivar uniqueness is now routinely performed, facilitating the cultivar release process, ensuring protection of outstanding selections in trials, and enabling rapid verification of identity during repropagation within the breeding program.

MAB has progressed over the last two years of this project from a promising means of improving breeding efficiency to a routine enhancer of various breeding operations in the WABP. The WABP applies DNA-based genetic markers in marker-assisted parent selection, parentage verification, assessment of crossing success, marker-assisted seedling selection on thousands of seedlings at a time, marker-assisted advanced selection description, genetic identity confirmation during repropagation, and fingerprinting of new cultivar releases – the first Rosaceae breeding program in the world to conduct routine MAB on so many fronts and the first for apple and related tree fruit for many of these applications.

Visits and discussions with apple breeders around the world have highlighted reasons for our success here. Foremost is the local industry support for the breeding program and its supporting science. A common feature elsewhere is that local industries are small, disconnected, and unclear about their cultivar and research needs. In some cases, breeding programs exist where local industries do not want or would not be able to adopt new cultivars. Another reason for our success is that there is a demand from the WA industry for fast, efficient return on investment in breeding. This demand has manifested in explicit requests for support from modern DNA-based technologies and expertise. In some other places, MAB is pushed upon breeders by molecular lab-based scientists, and in all places a huge chasm in understanding exists between genomics research and breeding application. From such requests has grown our apple genomics, genetics, and breeding team of advisors, technicians, and scientists. A third success criterion in our case is the focused perseverance of all involved.

Details on results

Obj 1. Establish a world-class long-term reference apple germplasm planting in Washington.

- 2041 trees are now in the Apple Germplasm Library (Figure 1), representing four germplasm “sets” (Parent, Pedigree, Mapping, and Diversity). 168 Parent Set trees planted in 2008 (67% of full capacity), 466 Pedigree Set trees planted 2008 (full capacity), 182 Mapping Set trees planted 2009 and 771 in 2010 (full capacity), and 227 Diversity Set trees planted 2010 (57% capacity).
- Library is spread across approximately 3.5 acres
- Several hundred trees planted in spring 2010 did not survive, primarily due to the Oct 2009 freeze event. These trees, primarily Mapping Set, will be replaced – providing the opportunity for a “staggered start” to improve the ability to identify true genetic effects.

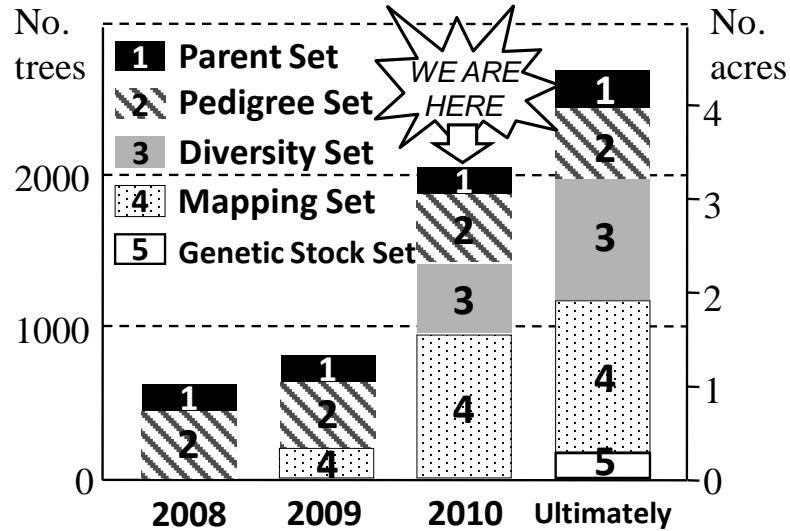


Figure 1. The Apple Germplasm Library will ultimately consist of about 2700 trees (with >2000 unique individuals) grouped into five Sets. The first planting was in spring 2008. The largest additions were planted in spring 2010. Maximum capacity will be reached gradually.

- Library's full capacity is about 2700 trees across 4.5 acres. Different components, with their varied uses, will be recycled at different rates in future years.
- NRI project used 7 cultivars of the Parent Set in 2009 season; most trees not yet fruiting then.
- RosBREED project is using 240 Pedigree Set seedlings and 24 Parent Set cultivars in 2010-2012.
- Flowering and fruiting dates and levels were recorded for all trees in 2009 and 2010.
- Parent Set trees used for crossing in WABP in 2010. Pedigree Set trees evaluated as Phase 1 seedlings in WABP in 2010.
- Sister plantings – “Apple Diversity Block”, “World of Apples” and “Student-Led Fruit Breeding Program” – for educational and public use was established in 2010 at the Tukey Research Farm in Pullman, using extra trees (after nursery propagation) of the Diversity Set and more than 800 *Malus sieversii* trees donated by G. Fazio, with planting conducted by undergrad and grad students and with financial support from the Dept Horticulture & Landscape Architecture.
- Presentations on Library in 2009: Good Fruit Grower article (“In search of superior apples”, pp14-15, July 2009), on-site – 1st Annual Sunrise Orchard Field Day (color 2-page flyer provided), on-site – 2009 annual Apple Crop Germplasm Committee meeting (color 2-page flyer provided).
- Presentations on Library in 2010: on-site – 2nd Annual Sunrise Orchard Field Day.
- Plan to evaluate fruit quality traits of Diversity and Mapping Sets and non-RosBREED Pedigree Set, alongside Parent Set cultivars, starting in the 2012 season, for enhanced genetic dissection, fishing for novel traits, and breeding application.
- Plan to evaluate insect resistance in Diversity Set in 2011.
- Plan to begin to populate the Genetic Stock Set in 2012 with seedlings from “fast breeding” (funded Technology Review project).
- Future funding requirements: ongoing funds for maintenance (currently approx. \$2000/acre/year) and for propagation and planting to fill capacity and replace dead trees. After 2011, plan to apply for federal funding to help offset these costs that to date have been borne by WTFRC.

Personnel involved: *Plant material choice:* C. Peace and B. Barritt, and J. Olmstead. *Provision of trees:* B. Barritt, K. Evans, P. Forsline, G. Fazio, Y. Zhu. *Planting:* Sunrise Research Orchard field crew, B. Konishi, L. Brutcher, J. Olmstead, K. Evans, C. Peace, CP's Pullman crew of T. Rowland, D. Edge-Garza, S. Haldar. *Plant maintenance:* Sunrise Research Orchard field crew, B. Konishi, L. Brutcher, J. Brunner (supervision). *Flyers:* C. Peace, J. McFerson.

Obj 2. Obtain comprehensive fruit quality phenotypic data on representative industry and breeding stock of Washington and the nation.

- Comprehensive fruit quality evaluations were conducted by the WTFRC crew on 130 cultivars and selections and 260 pedigree-linked seedlings growing in Washington and at the USDA-ARS apple collection in Geneva (Plant Genetic Resources Unit, PGRU).
- Measurements included harvest date, starch index, weight, crispness (instrumental Mohr Digi-Test Cn value and sensory 0-150 quantitative scale), firmness (instrumental Mohr Digi-Test penetrometer and sensory 0-150 quantitative scale), juiciness (sensory 0-150 quantitative scale), sweetness (instrumental for SSC), acidity (instrumental for TA), internal ethylene content (instrumental), and storage disorders (0-4 visual scale for decay, superficial scald, soft scald, shrivel, bitter pit, russet, lenticel disorders, watercore, internal browning, and greasiness). All traits were measured for each of 5 fruit at 3 evaluation times (harvest, 10 wks storage + 1 wk ripening, 20 wks storage + 1 wk ripening). Sensory evaluations were conducted by two panelists for each fruit.
- The WTFRC crew also measured SSC and TA for fruit juice samples of 190 cultivars, selections, and seedlings from the University of Minnesota breeding program (major NRI collaborator).
- The single-season dataset is being used, in combination with similar datasets from breeding programs of Minnesota, Italy, New Zealand, and Belgium (Figure 2), in our NRI project to refine understanding of the genetic control of apple texture and apply to industry and breeding.

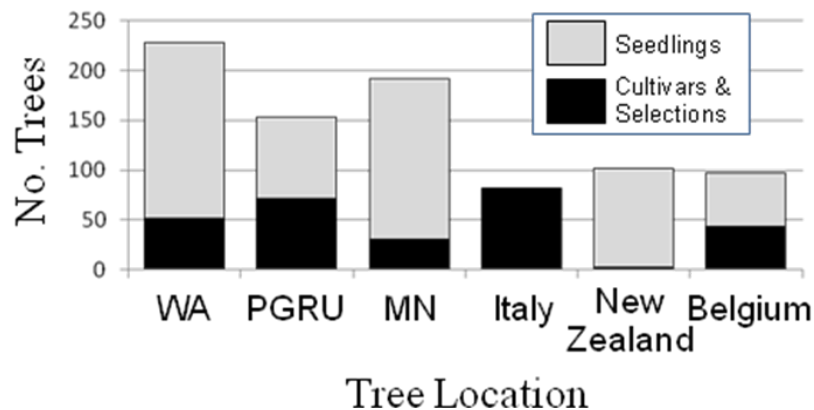


Figure 2. Scope of the fruit quality evaluation. A total of 861 apple cultivars, selections, and seedlings were evaluated, representing the apple breeding stock of the U.S. and the world. The WTFRC fruit lab crew conducted phenotyping for the samples from WA (WABP) and PGRU (Plant Genetic Resources Unit, USDA-ARS Geneva), as well as sugar and acid measurements for all samples from MN (Univ. Minnesota apple breeding program).

- Subsequent seasons (2010-2012) of fruit quality evaluation using many of the Parent Set and Pedigree Set trees of the Apple Germplasm Library are underway in the federally funded RosBREED project, enabled by the WTFRC investment in this 2009 effort.
- Future funding requirements: Fruit quality evaluation of remaining trees of the Parent and Pedigree Sets not covered by RosBREED is recommended for several seasons starting in 2012 (in combination with evaluation of trees of the Diversity and Mapping Sets).

Personnel involved: *Standardized phenotyping protocol development:* C. Peace, J. Luby, S. Brown, F. Costa, R. Dreesen, K. Evans, I. Hanrahan, P. Forsline, W. Guerra, J. Johnston, F. Laurens, J. Mattheis, J. McFerson, S. McKay, N. Oraguzie, J. Palmer, T. Schmidt, S. Tustin, E. van de Weg (that's a lot of experts to reach consensus with!). *WA germplasm choice:* C. Peace, K. Evans, B.

Konishi. *Harvest of WA fruit*: B. Konishi, K. Evans, WTFRC crew. *Harvest of NY fruit for WA evaluation*: P. Forsline, N. Gutkin. *WA fruit quality evaluations*: T. Schmidt, I. Hanrahan, M. Bell, F. Castillo, B. Konishi, K. Evans, J. Mattheis.

Obj 3. Ensure Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators.

- Washington fruit quality phenotyping is contemporary and coordinated with national and international collaborators. Standardized fruit quality phenotyping protocols for apple were developed in summer 2009 with an international network of experts. These protocols were also adopted in the RosBREED project in 2010.
- In Feb 2009, C. Peace visited three Plant and Food Research (PFR) stations on New Zealand's North Island (Auckland, Palmerston North, and Hawke's Bay), forging new opportunities for phenotyping technologies and standardization, molecular genetics, and functional genomics of apple fruit quality.
- In summer 2010, C. Peace visited the apple breeding programs of the University of Minnesota in St. Paul, MN, Cornell University in Geneva, NY (scion and rootstock), Katholieke Universiteit Leuven in Leuven, Belgium, and the Laimburg Research Centre for Agriculture and Forestry in Laimburg, Italy, in addition to NRI-funded visits to IASMA, Italy, and Plant Research International in Wageningen, Netherlands. Numerous discussions were also held at conferences with these and other apple breeders and germplasm curators of the world during the last two years regarding the status quo and future of apple breeding, fruit quality evaluation and genetics, MAB opportunities and challenges, and further prospects for collaboration.
- Future funding requirements: Nothing specific; we will continue to work with our collaborators in the U.S. and abroad on joint projects that amplify the WA apple industry's return on investment in genomics, genetics, and breeding research.

Obj 4. Enhance outreach efforts to demonstrate local impacts of genetic marker use for apple.

- The success stories of use of the ethylene genes and other genetic marker applications in the WABP were described at various venues with diverse audiences: WSHA annual conferences in 2009 and 2010, North Central Washington Apple Day 2010, Sunrise Research Orchard field days, United Fresh 2009 Convention in Las Vegas, NV, a popular science journal article (*Kean S. 2010. Besting Johnny Appleseed. Science 328: 301-303*), and Apple IAC/GGB (industry advisory committee / genomics, genetics, and breeding team) meetings, as well as recent WTFRC Apple Reviews.
- More outreach activities involving industry were planned from the outset, but the departure from WSU of co-PI J. Olmstead left us without a devoted Extension Specialist for this part of the project.
- Successes with the ethylene genes and other genetic marker applications in the WABP have also been described at many research venues in the last two years – most recently at the 5th International Rosaceae Genomics Conference in Stellenbosch, South Africa (C. Peace presented “DNA-informed breeding for high-impact fruit quality and productivity traits in Washington, USA”).
- Outreach efforts will continue to be integrated with the RosBREED project. Our local experiences with apple and sweet cherry have provided the major MAB examples to date. For example, the *Md-ACS1* ethylene genetic test for apple was used as the first of the “Jewels in the genome”, an ongoing article in the RosBREED quarterly Newsletter by RosBREED project director A. Iezzoni (described on p9 of Feb 2010 issue, which can be found at: www.rosbreed.org/resources/newsletters).
- Future funding requirements: Nothing specific. We will continue to take advantage of RosBREED resources and momentum to support outreach efforts in demonstrating and documenting local impacts of genetic marker use for apple.

Personnel involved: *Sunrise Field Days*: K. Evans, C. Peace, B. Konishi. *WSHA presentations*: K. Evans, C. Peace, and CP's Pullman crew of D. Edge-Garza, S. Haendiges, T. Rowland, S. Haldar, G. Lightbourn, S. Verma, and C. Starr.

Obj 5. Create a high-throughput seedling genotyping database for the WABP.

- Streamlined database not yet developed – awaits fruition of a collaboration with D. See and D. Main to hire a programmer for one year to develop the database and software tools. WTFRC funds already allocated here will provide \$10K, while D. See and D. Main will provide another ~\$45K.
- In the interim, we created a spreadsheet for automatic summarizing of data for managing genotypic data on several thousand WABP seedlings with the ethylene genes. This spreadsheet was used during seedling culling in spring and early fall of 2010.
- Marker-assisted seedling selection continues to be informed by our Seedling Selection Efficiency Tool (Excel spreadsheet). 2010 experiences used to update costs and durations involved.
- High-throughput seedling genotyping in 2011 will use the interim spreadsheet if necessary.
- Future funding requirements: D. Main seeks WTFRC funds in 2011 to develop high-throughput seedling genotyping database as extension of “breeders’ toolbox”. Development of RosBREED’s Breeding Information Management System in 2011-2013 will also include efforts in this area.

Personnel involved: *Databasing and software needs:* D. Main, D. See, K. Evans, C. Peace. *Interim spreadsheet:* D. Edge-Garza, T. Rowland, C. Peace, K. Evans.

Obj 6. Develop a DNA fingerprinting system for new cultivar releases from the WABP.

- An Excel spreadsheet-based calculator of cultivar parentage and uniqueness was developed. The system primarily uses simple sequence repeat (SSR) markers, which are excellent for such purposes, and also includes available data on functional markers such as the *Md-ACSI* and *Md-ACOI* ethylene genes for storability and SSRs near genes controlling other traits of interest.
- The system was applied to new cultivar releases from the WABP, ‘WA 2’ and ‘WA 5’ (Table 1; Evans KM, Barritt BH, Konishi BS, Dilley MA, Brutcher LJ, Peace CP. 2010. ‘WA 2’ apple. *HortScience* 45:668-669). The previously unknown pollen parent of ‘WA 2’ was determined to be ‘Gala’, and the parentage of ‘WA 5’ according to breeding records was confirmed (Table 1).

Table 1: Parentage analysis of ‘WA 2’ and ‘WA 5’ with nine apple genetic markers. Alleles inherited from ‘Splendour’, the mother of both ‘WA 2’ and ‘WA 5’, are shown in **bold**. The other allele must have therefore been inherited from their respective fathers (‘Gala’ for ‘WA 2’, ‘Co-op 15’ for ‘WA 5’).

WA cultivar	Parent cultivar	Marker genotype				
		<i>Md-ACSI</i> ^a	<i>Md-ACOI</i> ^b	<i>Md-Exp7</i> ^c	<i>Md-Exp2</i> ^c	<i>Md-PG2</i> ^c
‘WA 2’		2 :2	2 :2	202 :202	295 :450	359 :363
‘WA 5’		2 :2	1 :1	202 :214 ^d	295 :450	363 :363
	‘Splendour’	2:2	1:2	202:202	295:295	359:363
	‘Gala’	2:2	1:2	202:202	450:450	363:363
	‘Co-op 15’	1:2	1:2	202:214 ^d	450:450	358:363

WA cultivar	Parent cultivar	Marker genotype			
		Hi01b01 ^c	CH02b03 ^c	CH05c06 ^c	Hi04e04 ^c
‘WA 2’		162 :189	79 : 99	118 :126	216 :228
‘WA 5’		162 :162	79 : 107	104 :126	216:246 ^e
	‘Splendour’	162:189	99:107	104:118	216:246
	‘Gala’	189:189	75:79	118:126	228:246
	‘Co-op 15’	153:162	75:79	118:126	216:246

^a Allele 2 of *Md-ACSI* is associated with low ethylene production

^b Allele 1 of *Md-ACOI* is associated with low ethylene production

^c Allele size is measured in base pairs

^d Allele 214 is associated with scab resistance

^e Cannot determine which allele was inherited from ‘Splendour’

- Unique DNA profiles were established for both new cultivar releases. For the nine genetic markers reported, the probability of another tree having the same genotype as ‘WA 2’ is 1 in 8 million. For ‘WA 5’, this measure of uniqueness is 1 in 667 million (because ‘WA 5’ carries a rare allele associated with scab resistance).
- The DNA fingerprinting system was also used to ensure that during the repropagation of 23 WABP selections, to move them from TFREC to Columbia View, subsequent clones remain identical to their original mother trees.
- In another case on spring 2010, the system was used to sort out a nursery mix-up of several budded seedlings prior to Phase 2 planting.
- The system will continue to be used to establish uniqueness and verify or deduce parentage of advanced selections and new cultivar releases of the WABP, and for reliably tracking individuals through multiple rounds of propagation during breeding operations.
- Future funding requirements: None.

Personnel involved: *SSR choice:* C. Starr, C. Peace, E. van de Weg, G. Fazio. *Uniqueness and parentage spreadsheet:* C. Starr, C. Peace, S. Verma.

Obj 7. Continue to pipeline new markers for high priority traits into the WABP.

- For converting reported trait-specific markers into tools that can be used in the WABP, we use the 8-stage “MAB Pipeline” that is also the basis of enabling MAB for other Rosaceae breeding programs in the RosBREED project (www.rosbreed.org/breeding/community-breeders). RosBREED is currently improving the infrastructure underlying this Pipeline so that future efforts will be streamlined.
- The ethylene genes *Md-ACS1* and *Md-ACO1* are the first markers to be validated and converted into routine genetic tests for the WABP. This year, by spending \$10,000 on genetic screening, marker-assisted seedling selection provided an estimated net savings of \$62,000 in present and future costs for the WABP.
- Field planting in spring 2010 was eased after screening 2600 seedlings for *Md-ACS1* and *Md-ACO1*, which resulted in the culling of 1690 predicted inferior trees and avoids their future resource-consuming tree maintenance and fruit assessment.
- Integrating into an earlier stage of the breeding scheme, expensive nursery propagation and subsequent maintenance and assessment was avoided by culling 2900 seedlings (of 5300 screened in summer 2010) that carried inferior alleles for *Md-ACS1* and/or *Md-ACO1*.
- A marker for degree of skin blush (*Md-MYB1*) was assessed for utility to the WABP, with a resulting scientific publication (Zhu Y, Evans K, Peace C. 2010. Utility testing of an apple skin color *MdMYB1* marker in two progenies. *Molecular Breeding* 10.1007/s11032-010-9449-6). This marker may be used to guide crossing decisions in future years, but is unlikely to be used for seedling selection in the foreseeable future because seedlings with non-blushed fruit are not currently culled.
- A marker for crispness, acidity, and juiciness (targeting the *Ma* locus) was assessed for utility in the WABP using a meta-analysis of performance data for advanced and elite selections over the last decade. This marker guided crossing decisions since 2010, and will be used to cull seedlings beginning in spring 2011. This marker being investigated in greater detail in the RosBREED project, and outcomes will immediately inform WABP breeding decisions.
- A marker for firmness (*Md-Exp7*) is currently undergoing assessment of utility for the WABP. This marker has secondary use as a good indicator for apple scab resistance because it is closely linked – ‘WA 5’, for example, is probably scab resistant according to this marker.
- Several other markers are further back in the MAB Pipeline, including the *2MBAc* locus for “ripe apple flavor”. A Pullman-based PhD student, S. Verma, is investigating the interaction between extreme genotypes for this aroma-controlling locus and various genotypic categories of the ethylene genes for their joint ability to predict consistency of flavor after storage.

- Future funding requirements: About \$5000 annually for genetic screening consumables, with the availability of at least a part-time lab technician or student, will ensure that adequate attention is given to miscellaneous lab activities involved in Pipelining specific markers for the WABP.

Personnel involved: *Prioritization:* K. Evans, F. Bliss, C. Peace. *Genotyping efficiency:* D. Edge-Garza, T. Rowland, D. See, C. Peace. *Marker improvement:* D. Edge-Garza, S. Haendiges, D. See, C. Peace. *Validation and Utility:* C. Peace, E. van de Weg, M. Bink, Y. Zhu, K. Evans, S. Verma, Y. Guan. *MAPS:* K. Evans, C. Peace. *MASS cost-efficiency:* C. Peace, D. Edge-Garza, J. Olmstead, K. Evans, F. Bliss. *MASS trial use:* D. Edge-Garza, T. Rowland, B. Konishi, L. Brutcher, K. Evans, C. Peace. *New markers to enter the Pipeline:* C. Peace.

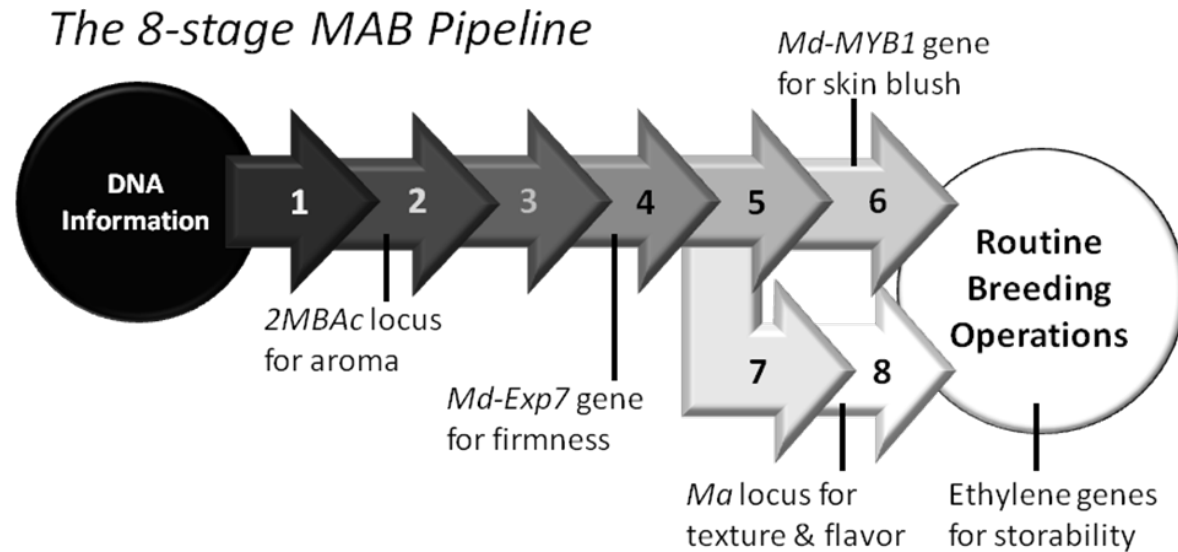


Figure 3: Current status of translating reported DNA information into routine applications in the Washington apple breeding program. The breeding “outlet” from stage 6 involves DNA-informed crossing decisions, while the breeding outlet from stage 8 is for seedling selection.

EXECUTIVE SUMMARY

Our 2009-2010 project on genetic marker assistance for the Washington apple breeding program (WABP) achieved many useful advances. Marker-assisted breeding (MAB) has progressed over the last two years of this project from a promising means of improving breeding efficiency to a routine enhancer of various breeding operations in the WABP. The WABP now applies DNA-based genetic markers to enhance breeding operations in several ways: Marker-assisted parent selection, parentage verification, assessment of crossing success, marker-assisted seedling selection on several thousand of seedlings at a time, marker-assisted advanced selection description, genetic identity confirmation during repropagation, and fingerprinting of new cultivar releases. The WABP the first Rosaceae breeding program in the world to conduct routine MAB on multiple fronts and the first for apple and related tree fruit for many of these applications.

Using markers for seedling selection is only one MAB application, as noted above, yet often attracts lots of attention because of the large numbers of seedlings and large amount of genotyping involved. However, we like to emphasize that using markers for wise parent selection is even more efficient. Our approach is that markers are only used for seedling selection when we are unable to choose parent combinations to avoid inferior seedlings in the first place.

The first markers to come out the application end of the “MAB Pipeline” (which is our systematic means of translating DNA information into practical tools for routine use) are those for the ethylene genes. The markers for the two targeted genes help predict fruit storability. These markers are used now in the WABP in parent selection, seedling selection, and to describe the genetic potential of new cultivars. However, the markers are applied on a case-specific basis. We do not intend for them to be used always, and the main outcome of our concurrent federally funded “NRI” project on apple fruit texture will be to refine their application based on the extent to which they can predict fruit quality. This prediction and utility depends on how they interact with other traits and the environment and how those traits meet industry demands. Thanks to the trail-blazing ethylene gene markers, more markers for other industry-prioritized traits are now coming into use.

Deliverables include:

- Apple Germplasm Library at the Sunrise Research Orchard established and used.
- Fruit quality evaluations on hundreds of breeding and collection trees successfully conducted.
- Protocols for standardized fruit harvest and quality evaluation developed and used.
- An international network of apple breeders, geneticists, collection curators, and fruit quality experts established and engaged.
- Success stories of first markers off the rank delivered to diverse audiences.
- Interim spreadsheet for automated management of seedling marker genotypes created and used.
- Cost-efficient seedling selection schemes using our Seedling Selection Efficiency Tool identified and conducted.
- Saved est. \$62K net from \$10K spent: spring field planting eased (1690 of 2600 seedlings culled); nursery propagation and future breeding costs avoided (2900 of 5300 seedlings culled).
- Unique DNA profiles for ‘WA 2’ and ‘WA 5’ to protect intellectual property rights established.
- Funding for multi-million dollar RosBREED project leveraged with WTFRC support.

Future Directions:

Evaluation, maintenance, and capacity-filling of Apple Germplasm Library; Open door for wider germplasm use in breeding and faster response to industry needs via fast-breeding methods; Improve release and adoption decisions about WABP’s new cultivars by revealing and communicating genetic potential for commercial performance; Enhance and utilize bioinformatics support for maximized access to performance and DNA-based data; Establish a full-time genotyping technician/manager in Pullman; Coordinate with RosBREED project to maximize WA tree fruit industry benefit.