**FINAL PROJECT REPORT** **YEAR**: 3 of 3

**Project Title:** Apple scion breeding


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|---|---|---|---|
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**Total Project Funding:** **Year 1**:$169,910 **Year 2:**$239,628 **Year 3:**$203,217

**Other funding sources: None**


**Budget 1History:**
**Organization Name:** WSU-TFREC **Contract Administrator:** ML Bricker and Kevin Larson
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| Item | 2009 | 2010 | 2011 |
|---|---|---|---|
| **Salaries**[1] | 60,540 | 50,352 | 55,005 |
| **Benefits** | 23,005 | 16,616 | 19,792 |
| **Wages**[2] | 15,500 | 20,000 | 20,800 |
| **Benefits** | 2,790 | 2,960 | 3,120 |
| **Equipment** | 50,000 | 0 | ,0 |
| **Supplies**[3] | 0 | 19,700 | 17,500 |
| **Supplies**[4] | 0 | | 8,800 |
| **Travel** | 14,200 | 15,500 | 16,900 |
| **Plot Fees** | 0 | 0 | 0 |
| **Total** | 166,035 | 125,128 | 141,917 |

**Budget 2 History:**
**Organization Name:** Willow Drive **Contract Administrator:**

| Item | 2009 | 2010 | 2011 |
|---|---|---|---|
| **Trees** | 700 | 6000 | 0 |
| **Phase 2 trees**[1] | 700 | 6,000 | 2,000 |
| **Phase 3 trees**[2] | 0 | 0 | 8,300 |
| **Seedling propagation**[3] | 3,175 | 108,500 | 51,000 |
| **Plot Fees** | 0 | 0 | 0 |
| **Total** | 3,875 | 114,500 | 61,300 |

## OBJECTIVES

1. Produce, through traditional breeding methods, promising selections and subsequently elite selections with outstanding eating quality and commercial potential.
2. Use extensive trait phenotyping in combination with genomic tools (phenotype/genotype associations) to develop DNA-informed seedling selection for key fruit and tree traits.
3. Use both objective (instrumental) and subjective (sensory) evaluation techniques to identify selections with outstanding eating quality.

## SIGNIFICANT FINDINGS

1. An Industry Advisory Council (IAC) for the WSU apple breeding program (WABP) was established in 2009. IAC visits have included the Phase 1 seedling evaluation orchards and a Phase 2 planting as well as the Phase 3 grower trials. The IAC actively participated in developing evaluation and commercialization guidelines for WSU apple releases together with WSU and the WTFRC.
2. Thirty-six new crosses were made and approximately 57,000 seeds were produced in the three years of this project. Seedlings from approximately 52,000 germinated seeds from 2008-2010 crosses were grown in the greenhouse, more than 11,000 of which were screened for fire blight resistance.
3. Approximately 12,500 seedlings were screened using DNA markers for long-storage ability and fruit texture; only pre-selected seedlings were then propagated on M.9 for orchard evaluation. More than 27,000 seedling/M.9 trees were subsequently produced in the nursery for planting in Phase 1 seedling orchards within the three years of this project.
4. DNA-informed seedling selection was also used to pre-select for long-storage ability and texture in more than 2,500 seedlings from 2006 immediately prior to planting in the Phase 1 selection orchard in 2010.
5. Promising selections in Phase 2 trials (planted in 2004, 2005, 2006, 2007, 2008 and 2009) at three trial sites in Central Washington were evaluated for productivity and fruit quality.
6. Forty-six new promising selections were planted in Phase 2 trials at three trial sites within the three years of this project (2009, 2010 and 2011).
7. Bar-coding technology has been incorporated into the fruit laboratory and orchards (Phases 1 and 2) to facilitate sample tracking and reduce possible transcription errors.
8. One new elite selection was propagated for Phase 3 trials in 2010.
9. Samples of fruit from eleven Phase 2 and 3 selections from various sites and pick dates were profiled by the sensory panel in Pullman and rated for consumer acceptance within the three years of this project.
10. Four members of the TFREC breeding team received sensory panel training at WSU-Food Science and Human Nutrition under the supervision of Dr. Ross.
11. The first two new apple varieties have been released from the WABP following guidelines developed by the IAC. ‘WA 2’ was released in 2009 (U.S. patent # PP21,710 P3) and ‘WA 5’ in 2010 (patent-pending). A third release has been approved by WSU’s Cultivar Release Committee, collection of patent data is complete and trees began large-scale propagation in 2011.
12. DNA marker development has progressed well. The WABP became one of 12 “demonstration breeding programs” with the start of the multi-million dollar SCRI-funded project, RosBREED. A reference germplasm set for identifying and refining new DNA tests was established that includes more than 250 cultivars and seedlings at the Sunrise Research Orchard. Since 2010, this Sunrise germplasm has been comprehensively phenotyped and genotyped in the RosBREED project, to be combined with similar data from other U.S. apple breeding programs for more powerful analyses. A DNA marker for skin color was published by Dr. Zhu with Drs. Peace and Evans.

## RESULTS & DISCUSSION

The aim of the WABP is to produce a portfolio of new improved unique varieties, especially selected for the growing environment of central Washington. The reporting years of this project have seen significant leaps forward in achieving this aim: 1) the release of Washington's first apple varieties bred and selected specifically for its growing conditions; and 2) the first use of routine marker-assisted selection for fruit quality not only for this program but also any woody perennial fruit crop worldwide.

### Apple Releases

The efficiency of the program compared to other apple breeding programs was highlighted by the release of the first variety only 15 years from the cross (a more typical timeframe being 18+ years).

'WA 2' was released in 2009 from an open-pollinated 'Splendour' seedlot created in 2004 ('Gala' was later identified via DNA markers as being the father). This first WABP release is a compact, precocious tree with no reported problems of sunburn, bitter pit or mildew. Fruit harvest is 'Red Delicious' season after which fruit quality is improved by a few months of storage. Storage tests to date have shown it has excellent potential for long term storage (9+ months) without the need for 1-MCP.

'WA 5' was released in 2010 from a 'Splendour' x 'Coop15' cross also made in 1994. It has a similar tree habit to 'WA 2' but is earlier in season ('Golden Delicious' season) and eats well straight off the tree. 'WA 5' may benefit from 1-MCP application although storage tests are still underway.

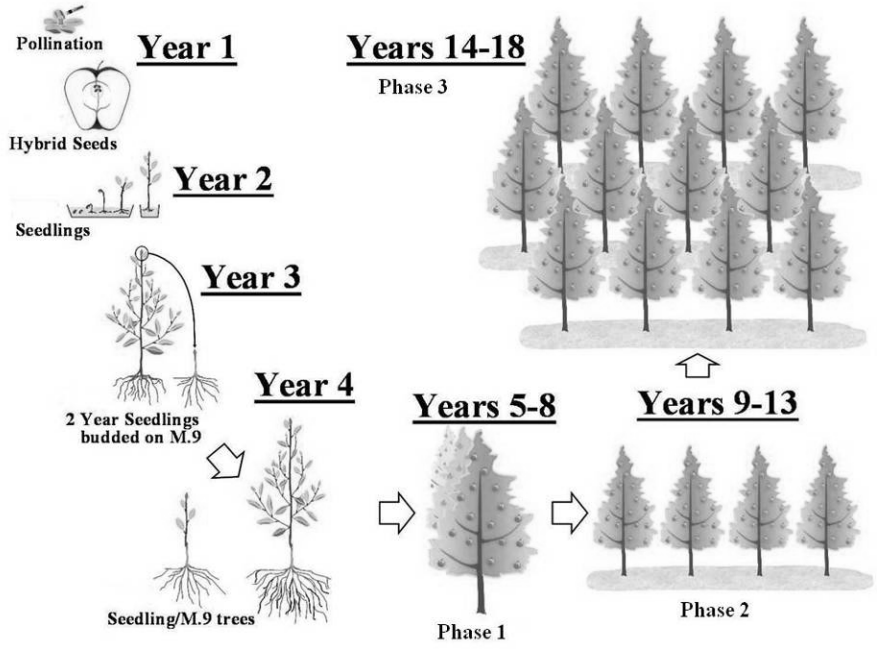
A U.S. Plant patent was approved for 'WA 2' in 2011; the patent for 'WA 5' is pending. The WTFRC is the licensee for both varieties and the release strategy was developed in collaboration with the WSU Research Foundation, with guidance from the apple Industry Advisory Council (IAC) chaired by Brent Milne. Release of both varieties in the U.S. is restricted to Washington State growers.

**Marker-assisted breeding**
In 2010, the WABP was the first apple breeding program in the world to implement DNA markers to routinely pre-screen seedlings for fruit quality. Seedlings in the nursery were screened with allelic tests for two genes involved in ethylene production (*Md-ACS1* and *Md-ACO1*) that indicate genetic potential for storability. Seedlings with the least favorable allelic combinations (genotypes) were culled before planting in Phase 1 orchards. Nursery charges for 2012 will reflect this reduction in seedlings propagated and maintained. The benefit:cost ratio of using DNA markers was estimated at more than 5:1.

In 2011, a third genetic test was added to the pre-screening, for the *Ma* locus associated with acidity, crispness and juiciness. The test was used to screen seedlings at the earliest stage in early spring in the greenhouse. To facilitate timely execution of genetic screening, we secured the part-time services of a full-time genetic screening technician in the Peace laboratory, Terry Rowland.

Ethylene gene predictive genotypes for potential parental varieties and selections have been used in the WABP for several years to determine optimal cross combinations. The 'pool' of parental germplasm was routinely tested with all available predictive markers as they were developed. Progenies from the breeding program were also used to develop a new apple skin color marker by Dr. Zhu.

**General project update**
The WSU apple breeding program has continued to generate new progenies either by inter-crossing the best of its selections or by crossing these selections with new parental germplasm. DNA information now plays a significant part in choice of cross combination, and this information is continually updated for common parents and selections as new markers are developed. The addition of this type of data to the breeding program database is a powerful tool, enabling the breeder to search for superior genetic potential when developing a crossing scheme or selecting seedling germplasm to move forward.




Washington Apple Breeding Program schematic

In 2010 and 2011, several newly germinated seedling progenies were inoculated with fire blight to identify resistant individuals. All other progenies not being marker-screened received a basic screen for resistance to mildew. A new genetic marker for fire blight resistance was developed for WABP germplasm in late 2011 and will be incorporated into parent selection decisions from 2012.

Considerable effort has been made towards improving the logistics of sampling seedlings for genetic screening. Spring 2012 will see the first large-scale test of our new '96-pot' format that mimics the '96-well' molecular laboratory format. This change should reduce opportunities for sampling error and increase confidence in accurately connecting laboratory results to the correct seedlings whilst minimizing labeling.

*Phase 1*

More than 27,000 trees were propagated onto M.9 rootstock and planted into Phase 1 orchards within the timeframe of this project. Two key changes to standard protocol were initiated during this time: all plots were fumigated prior to planting and trees were given bar-coded labels to facilitate sample tracking in the fruit analysis laboratory. The use of a hand-held datalogger and mini-printer enables fruit samples to be given bar-coded labels in the orchard; these labels stay with the samples throughout storage and laboratory evaluations. Juice samples for soluble solid concentration and titratable acidity are also bar-coded. The fruit analysis laboratory was partially equipped using funding from this project. The Mohr® DigiTest, the digital refractometer and the autotitrator all accept scanned bar-codes as samples IDs and, once measurements are completed, output data in an easily accessible format that is readily transferred into Excel spreadsheets formatted for streamlined data entry to the new Breeders Toolbox database. This system greatly reduces the possibility of sample mixing and data entry errors as well as saving a considerable amount of data entry time.

New selection fruit samples were harvested as close to Cornell starch stage 3 as possible, to standardize "commercial maturity", and stored for two months in regular atmosphere 34$^{o}$F before being evaluated. 'Keepers' (selections that rated well in earlier years) were harvested up to four times in weekly intervals (crop-load permitting), with at least one harvest being before Cornell starch stage 3, and each sample was tested both at harvest and after two months in regular atmosphere at 34$^{o}$F. 'Keepers' that continue to rate well are propagated for Phase 2 trials. In 2011, G41 was used for propagation rather than M.9 to reduce the need for fumigation of future plantings.

*Phase 2*

During the course of this project, two new Phase 2 sites have been established. The WSU Columbia View orchard was replaced by one at Sunrise and the Basin City orchard was replaced by a new orchard adjacent to the Phase 3 block in Prosser. The bar-code labels have also been adopted in the Phase 2 orchards, easing sample tracking through the laboratory. Fruit samples were harvested similarly to the Phase 1 'keepers' but, as more fruit is available, samples were also taken for extended storage (four months in regular atmosphere at 34$^{o}$F). The dataloggers are also now linked to calipers for trunk diameter measurements to reduce another source of transcription error.

Phase 2 fruit was tasted and rated by industry members at each of the three annual Washington State Horticultural Association annual conferences within the duration of this project. Four Phase 2 selections were tested by Dr. Ross's trained sensory panel and rated by consumers in 2009. Of these, one has now been propagated for Phase 3 trials.

*Phase 3*(managed in collaboration with the WTFRC [Auvil & Hanrahan])
Advice and regular visits from the IAC has led to development of thinning recommendations for crop-load management in Phase 3 trials. Harvest dates were determined by the WTFRC using a combination of starch level and color break with a typical three to four picks per selection per site (crop-load permitting). Small samples of each have been evaluated through the fruit evaluation laboratory. As the volume of fruit increased, more detailed storage testing were undertaken by the WTFRC, with the protocol in 2011 including post-harvest drenching, 1-MCP treating of some fruit and both regular and controlled atmosphere treatments for up to eight months. Some samples have also been run over a packing line including a wax application.

Two Field Days were hosted in 2010 (Quincy and Brewster) and one in 2011 (Quincy) to give growers the opportunity to see the Phase 3 plantings, particularly 'WA 2' fruit on the tree.
Four Phase 3 selections were tested by Dr. Ross's trained sensory panel and rated by consumers in 2009 with a more detailed analysis of two of them (pick dates and sites) in 2010. This was followed in January 2011 by development of a sensory profile of five standard varieties to help establish a baseline and identify gaps in the current market selection of varieties.

One new selection was added to the Phase 3 plantings in 2009, bringing the total up to ten. Of these, the IAC recommended discontinuing the evaluation of three selections (WSU 7, 48 and 49) as potential licensable cultivars by the WTFRC or by its soon-to-be-established Management Entity. A further selection was found to be virus-infected so was withdrawn from Phase 3. A decision was made in 2011 by the WTFRC to disengage from 'WA 5'.

It became apparent in 2011 with the increased volume of fruit now present that detailed evaluations of all remaining Phase 3 selections each year would be unmanageable. Priorities were agreed upon and the team focused on a manageable number of elite selections in 2011, taking only a smaller sample of lower priority selections. A new site was planted with one selection in Mattawa in 2011. Due to a change of ownership of the Brewster site, a new northern site is being sourced. One new selection has been propagated for planting in Phase 3 in 2012, an early-season apple of 'Honeycrisp' parentage. One elite selection from the 2008 plantings was propagated in 2011 with a view to commercial Phase 4 release in winter 2012. A successful application was made to WSU's Cultivar Release Committee in 2011. Patent information was collected and an application is in preparation.

## EXECUTIVE SUMMARY

The aim of the Washington State University Apple Breeding Program is to produce a portfolio of new improved unique varieties, especially selected for the growing environment of central Washington. The reporting years of this project have seen significant leaps forward in achieving this aim: 1) the release of Washington's first apple varieties bred and selected specifically for its growing conditions and 2) the first use of routine marker-assisted selection for fruit quality not only for this program but also any woody perennial fruit crop worldwide.

Washington State growers are now able to grow 'WA 2' commercially; a number of growers applied for licenses at the first opportunity in 2010. When originally planned, it was expected that the full cycle of Phases 1 to 3 of the breeding program would take 18 years (considerably shorter than other apple breeding programs) which would mean that the first release would be expected in 2012. Not only was this timing reduced, but the development and application of marker-assisted breeding has greatly enhanced this program's ability to develop further superior varieties in the future.

Within the timeframe of this project, the WABP has set up a new fruit analysis laboratory, sourcing equipment (partially funded by this grant) that enables efficient analysis and reduced data entry effort and error. Sample tracking fruit from the orchard to the laboratory has been streamlined with a bar-coding system that is compatible with fruit evaluation equipment. Significant progress has also been made in streamlining the logistics of routine marker-assisted seedling selection; an area frequently over-looked but potentially a huge source of error. Seedlings will be planted using a new 96-pot system in January 2012.

Deliverables:

1. Two new apple cultivars released to the Washington industry.

2. One further apple cultivar propagated for release in 2012.

3. World-first for integrating a potentially revolutionary new technology (marker-assisted breeding) into routine breeding operations – an outcome of the Technology Roadmap.