

FINAL PROJECT REPORT

Project Title: Apple scion breeding program

PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-663-8181 x245
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI (1): Cameron Peace
Organization: WSU-Horticulture
Telephone: 509-335-6899
Email: cpeace@wsu.edu
Address: PO Box 616414
City/State/Zip: Pullman/WA/99164

Cooperators: Bruce Barritt, Professor Emeritus, WSU; Yanmin Zhu, USDA-ARS Wenatchee; Amit Dhingra, Dorrie Main, Carolyn Ross, WSU Pullman; Tom Auvil, Ines Hanrahan, WTFRC; Roger Adams, Willow Drive Nursery, Ephrata; Craig Hardner, Australian Crop Genetic Services, Brisbane, Australia; Fred Bliss, Davis, California.

Other funding sources

Agency Name: WTFRC Apple Review

Amount awarded: \$59,740 (2012-2013)

Notes: "Further development of an online toolbox for tree fruit breeding" PI: Main. Co-PIs: Evans, Jung, Peace, Oraguzie. Establishment of bioinformatics and databasing support to facilitate the translation of genomics information into application in WSU tree fruit breeding programs. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: WTFRC Apple Review

Amount awarded: \$89,963 (2012-2013)

Notes: "Increasing decision confidence in cultivar development and adoption" PI: Peace. Co-PIs: Hardner, Evans, Bink. Synergistic project to improve release and adoption decisions about the WABP's new cultivars.

Agency Name: WTFRC Technology Review

Amount awarded: \$23,777 (2012-2013)

Notes: "Breeding in the 21st century: Technology platform for fast breeding" PI: Dhingra. Co-PI: Evans. Synergistic project to implement fast-track breeding to elite selections from the WABP.

Agency Name: WTFRC Apple Review

Amount awarded: \$77,724 (2013-2014)

Notes: "Support systems to deliver elite new cultivars for extended storage" PI: Peace. Co-PIs: Hardner, Evans. Synergistic project to support release and adoption decisions about the WABP's new cultivars.

Agency Name: WTFRC Apple Review

Amount awarded: \$22,942 (2013-2014)

Notes: "Reduction of generation cycle in apple breeding" PI: Dhingra. Co-PI: Evans. Synergistic project to implement fast-track breeding to elite selections from the WABP.

Agency Name: WTFRC Apple Review

Amount awarded: \$36,254 (2013-2014)

Notes: "WA 38 rootstock and systems trial" PI: Evans. Co-PIs: Lewis, Musacchi. Synergistic project to develop systems for WA 38 production.

Agency Name: WTFRC Apple Review

Amount awarded: \$23,240 (2011-2014)

Notes: “‘WA 2’ plant variety rights applications” PI: Evans. Co-PI: Kelly. Synergistic project to implement PVR protection for ‘WA 2’.

Agency Name: WTFRC Apple Review

Amount awarded: \$269,000 (2014-2016)

Notes: “After RosBREED: Developing and deploying new apple DNA tests” PI: Peace. Co-PIs: Hardner, Evans, Main. Synergistic project to develop new DNA tests for the WABP and effectively use outcomes of many DNA tests into routine selection decisions.

Agency Name: WTFRC Apple Review

Amount awarded: \$85,479 (2012-2014)

Notes: “Incorporating fire blight resistance into Washington apple cultivars” PI: Norelli. Co-PIs: Evans. Synergistic project to identify sources of resistance to fire blight.

Agency Name: USDA-CSREES Specialty Crop Research Initiative

Amount awarded: \$2.0 million + equal matching from universities, industry (Sep 2009 – Aug 2014)

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 Crop Research Initiative

Amount awarded: \$7.2 million + equal matching from universities, industry (Sep 2009 – Aug 2014)

Notes: “RosBREED: Enabling marker-assisted breeding in Rosaceae”. PI: Iezzoni. Co-PIs include Peace, Main, and Evans. To establish sustainable DNA-informed breeding for U.S. Rosaceae crops.

Agency Name: USDA-CSREES Specialty Crop Research Initiative

Amount awarded: \$2.7M + equal matching from universities, industry (Sep 2014 – Aug 2019)

Notes: “Genome Database for Rosaceae: Empowering specialty crop research through big-data driven discovery and application in breeding”. PI: Main. Co-PIs include Evans and Peace. Synergistic project for application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES, Specialty Crop Research Initiative

Amount awarded: \$10.0 M (Sep 2014 – Aug 2019)

Notes: “RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars”. PI: Iezzoni. Co-PIs include Peace, Evans, and Main. To lift DNA-informed breeding tools and strategies to the next level for U.S. rosaceous crops.

Total Project Funding: Year 1: \$317,461 Year 2: \$305,237 Year 3: \$239,563

Budget History:

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	18,830	18,830	0
Benefits	6,626	7,232	0
Wages	52,500	47,250	10,548
Benefits	7,650	6,885	7,032
RCA Room Rental	8,400	8,400	8,400
Shipping	0	0	0
Supplies	3,000	3,000	0
Travel	6,875	7,000	5,000
Plot Fees	0	0	0
Total	103,881	98,597	30,980

Footnotes:

¹ Lab volume decreased by 50%, Field by 30%.

² Lab and Field volume decreased by 10%.

³ In-state travel to research plots which are spread out across the state.

Budget History 1

Item	2012	2013	2014
Salaries	54,642	56,828	57,035
Benefits	21,694	22,562	22,106
Wages	20,800	21,632	22,113
Benefits	3,099	3,223	4,179
Orchard establishment supplies	19,000	20,000	19,000
Genotyping supplies	11,000	13,000	15,000
Travel	15,000	15,000	15,000
Plot fees	8,400	8,800	8,800
Total	153,635	161,045	163,233

Budget History 2

Item	2012	2013	2014
Seedling propagation	34,720	42,095	41,850
Phase 2 trees	4,425	3,500	3500
Phase 3 trees	20,800	0	0
Plot Fees	0	0	0
Total	59,945	45,595	45,350

Objectives:

1. Produce, through conventional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and commercial potential.
2. Using both objective (instrumental) and subjective (sensory) evaluation techniques to develop selections with outstanding eating quality.
3. Use extensive performance evaluation in combination with genetic markers to validate and implement new DNA tests for key fruit and tree traits.

Significant Findings:

1. Fifty-five new crosses were made in this project producing approximately 86,000 seeds.
2. Seedlings from approximately 50,000 seeds were grown in the greenhouse.
3. Approximately 22,750 seedlings were screened with DNA markers for fruit quality; just over 11,750 were culled leaving the remaining to be transplanted to Willow Drive nursery.
4. The final count of new Phase 1 trees planted in this project was approximately 13,000.
5. Promising selections already in Phase 2 trials (planted in 2006, 2007, 2008, 2009, 2010, 2011 and 2012) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
6. Bench grafted plants of 43 selections were tested in the greenhouse for resistance to fire blight in 2013 and 2014. Six selections were as resistant as Red Delicious with several more showing some level of field tolerance.
7. Fifteen new promising selections were planted at three evaluation sites in Phase 2 trials in this project. Trees planted in 2013 onwards were on Geneva 41 rootstock.
8. Phase 3 has been considerably streamlined, resulting in a reduction in the budget. Two hundred and forty trees of WSU 46 were shared between three Phase 3 sites in spring 2012. WSU 68 was advanced to Phase 3 at the Quincy site by top-working on trees *in situ* in 2013. WSU 64 & 65 (on Geneva 41 rootstock) are due to be planted at the Quincy site in spring 2015.
9. A Cultivar Licensing Committee was formed that developed a release strategy for WA 38 and provided a framework for future releases from the WABP.
10. WA 38 performed well in comparison to 'Honeycrisp' in a series of three consumer testing sessions (October, December and March 2013/14).
11. New genomics discoveries of value to the WABP were made within the RosBREED project, and developed into additional trait-predictive DNA tests.
12. Trait-predictive DNA information was obtained for all prospective parents using nine DNA tests – for storability, crispness, juiciness, firmness, acidity, skin color, and bitter pit incidence and used to support crossing decisions.
13. Trait-predictive DNA information was also obtained for all selections advancing into Phase 2.
14. Parentages of WABP parents and selections were confirmed, refuted and deduced, improving our understanding of the breeding value of certain parents.
15. Genetic identity was confirmed for all mother trees of WA 38 planted in the nursery mother tree blocks. All trees tested as true to type using several DNA markers.
16. The patent for WA 38 was approved.

RESULTS & DISCUSSION

Objective 1 - Breeding:

Crossing in 2012 through 2014 illustrates some of the challenges commonly faced in the breeding program. While the same amount of effort went in to making crosses in each of the three years, the number of seeds produced ranged from 45,284 in 2012 to just 9,564 in 2013 due to the weather through bloom time. Surplus 2012 seeds were 'banked' for years such as 2013; this allowed a degree of consistency over the number of seeds sown each year which helps with long-term planning in the program.

Seedlings from approximately 50,000 seeds were grown in the greenhouse during this project. Almost half of those seedlings were screened with one or more DNA markers for fruit quality using the now routine “96-plant” format. This format, developed within the WABP, to mirror the DNA lab’s “96-sample” (12x8) plate system, greatly reduces the possibility of data transfer and labelling errors which would otherwise reduce the confidence in the DNA test results.

Greenhouse trials were established to explore options for different growing systems for pre-selected seedlings (post-DNA testing). Once sufficient DNA tests are available to cut the numbers of individuals in specific progenies dramatically, growing the seedling in a pot in the greenhouse before hardening off outside should result in sufficient propagating buds to either move forward straight to Phase 2 or perhaps a Phase 1.5.

Three thousand seedlings were screened for resistance to fire blight in the greenhouse and then re-tested in the field the following year. Due to the retirement of Dr. Larry Pusey from the USDA-ARS facility in Wenatchee, the breeding team learned how to prepare fire blight inoculum and using a protocol from Dr. Ken Johnson (O.S.U) has transitioned to the use of self-prepared freeze-dried inoculum.

Approximately 13,000 new Phase 1 trees were planted at the TFREC Columbia View orchard for evaluation within this project. Older blocks of seedlings were removed annually and selected mother trees replanted into the Sunrise orchard parent block to facilitate land preparation for future plantings. Selection in the orchard was particularly difficult in 2012 with the high level of smoke in the orchards and had to be based primarily on fruit appearance with eating quality tested back in the lab. There was considerable hail damage in the Phase 1 and Phase 2 blocks at Sunrise in 2013, however, there was still sufficient fruit to enable Phase 1 selection. Additional harvesting from Phase 1 by the two graduate students (Julia Harshman and Paul Sandefur) as part of the aligned project “Support systems to deliver elite new cultivars for extended storage” (Peace, Hardner and Evans) has maximized the utility of those blocks.

Forty-three Phase 2 & 3 selections were tested for fire blight resistance using shoot inoculation of bench-grafted trees grown in pots in the greenhouse in 2013 and 2014. Considerable differences were seen in the levels of resistance among the selections with several Phase 2 selections appearing to be as highly resistant as ‘Red Delicious’.

Two hundred and forty trees of WSU 46 were shared between three Phase 3 sites in spring 2012. Storage trials continued on fruit from six other elite selections harvested in 2011 (conducted by I. Hanrahan). Selections WA 5 and WSU 19 were discontinued due to internal breakdown issues in storage. WSU 17 and 36 were rejected from Phase 3 due to fatal flaws (fruit splitting and tree health, respectively). Original Phase 3 plantings included too many selections and were consequently expensive to evaluate fully. This phase has been re-organized to make it efficient but yet affordable with a steady progression of selections rather than moving too many forward together.

The first fruit were harvested from the latest P3 selection (WSU 46) in 2013, two new selections were propagated for planting in spring 2015 (WSU 64 and 65) and a further new P3 selection (WSU 68) was top-worked onto rejected WSU 17 trees at the Quincy site in 2014. Wood of WSU 64, 65 and 68 was all sent to the Clean Plant Center, Prosser in 2014 to initiate virus testing.

One hundred and seventy new trees of WA 38 were top-worked onto rejected P3 trees in the Quincy and Prosser Phase 3 sites. The trees have grown well and are now producing useful fruit.

Following its release by WSU's Cultivar Release Committee, the patent application was submitted for WA 38 and was approved in September 2013. Certified virus tested material was established in most Washington nurseries and has all been fingerprinted in the Peace lab to confirm correct labelling. A release strategy for WA 38 was finalized by Jim Moyer (WSU ARC Director) with advice from the recently formed Cultivar Licensing Committee. This strategy was described in the *Good Fruit Grower*, at the NNII December meeting and also at the Hort Show and has now been followed through with the first 300,000 trees being allocated through a drawing in 2014 (for planting 2017).

Objective 2 - Sensory/instrumental evaluation:

Bar-coded fruit samples continued to be of benefit in reducing sample mixing and data-entry errors. The fruit testing protocol was changed slightly in 2012 onwards. Data analysis from the supporting WTFRC-funded project "Increasing decision confidence in new cultivar development and adoption" showed that Phase 2 selections ranked in the same order of acceptability at harvest as after two months storage. Consequently, to reduce tasting fatigue, only instrumental tests were performed on these samples at harvest, saving considerable time and effort during the busiest season of the program. Also after the typical two months of air storage, fruit samples were stored for a further week at room temperature before sensory and instrumental evaluation was performed; a more severe screening than previous years but one more reflective of the final consumer experience.

Data analysis using the Elite Advance software developed in the above mentioned project is now routinely used with the Phase 2 fruit data. Together with knowledge of the overall performance of the selection, it is useful in determining which advanced selections should be promoted to elites.

Fruit from eight Phase 2 selections were sent to the Ross lab, Pullman for trained sensory panel evaluation and consumer panel evaluation in spring 2012 as part of the WTFRC-funded project "Sensory and consumer acceptance of advanced apple breeding selections". The Ross lab has also been instrumental in organizing pairwise consumer tests of WA 2/Gala, WA 38/Gala and WA 38/Honeycrisp and analyzing the subsequent data. The WSU selections performed very well in each case and data has been published in the *Good Fruit Grower* and presented at various meetings.

In order to try and improve WABP's ability to harvest fruit from seedlings at optimum maturity, Julia Harshman (Evans graduate student) is using Phase 2 fruit to test the potential of the DA meter (T.R. Turoni, Italy) for determining harvest maturity. The results appear to vary depending on the skin and flesh color (white/cream/green hues) of the apples; analysis of the data from the 2014 harvest is still on-going.

Objective 3 - DNA-informed breeding tools:

DNA tests targeting numerous genomics regions influencing valuable traits were developed and validated for use on WABP germplasm. DNA information from these trait-predictive DNA tests was subsequently used to support breeding decisions for greater efficiency, accuracy, creativity, and pace in developing new cultivars for Washington. Major advances in this technology were made possible by leveraging industry support with federal grants, especially the first RosBREED project (2009-2014) and the bioinformatics-based Tree Fruit GDR project (2009-2014).

New DNA tests were developed and applied to the WABP for several valuable fruit quality traits. Prior to this project, DNA tests for fruit storability ("ACS" and "ACO" ethylene genetics) and crispness and juiciness were in routine use for parent and seedling selection. During this project, further DNA tests were developed, adapted, and refined for the WABP and put into use in parent selection, seedling selection, and characterizing promising selections. These further DNA tests could explain some of the variance for the traits of firmness at harvest and after storage ("PG1"), skin color,

and, all from a single DNA test at one particular genomic region, acidity, overall superior texture after storage, bitter pit incidence, size, greasiness after storage, sweetness via sucrose content, and calyx opening.

DNA information was gathered and used on the WABP's diverse portfolio of parents and promising selections. This information guided crossing decisions, helping target the creation of seedling families enriched for valuable genetics. The need for using the original ACS and ACO tests on seedlings has reduced in recent years because (1) crosses made have used ACS/ACO information to help avoid seedlings that generate inferior genotypes, and (2) new DNA tests with improved predictiveness are now available. Using the high-resolution RosBREED datasets, parentages of cultivars and selections have been confirmed, refuted, and deduced (note: WA 2 and WA 38 were re-confirmed as having the parentage previously reported: WA 2 = Gala × Splendour, WA 38 = Enterprise × Honeycrisp). Knowledge advancements include revelations about grandparents and great-grandparents of WA cultivar releases. Confidence in parentage records improved appreciation of the breeding value of certain parents (and refuted the value of others). Full marker characterization of selections increased decision confidence in advancing material into Phase 2 and out of Phase 2 toward commercial release.

During this project, new upstream genomic discoveries were made by our RosBREED-funded graduate students relying on RosBREED datasets. The students focused on sweetness (Yingzhu Guan) and acidity, crispness, and juiciness (Sujeet Verma). Their discoveries have already led to development of two new DNA tests by another of our graduate students (Paul Sandefur) and an undergraduate intern (Ashley Powell) in the 2014-2016 WTFRC project "After RosBREED: Developing and deploying new apple DNA tests". These new DNA tests are for fructose content and acidity (which incorporates the two major acidity-influencing regions in the apple genome). Another graduate student (Feixiong Luo) is preparing DNA tests for powdery mildew susceptibility and pink vs. white flesh color for application in 2015.

WABP Publicity

Gift boxes of WA 2 and WA 38 were delivered to numerous industry contacts throughout the project with follow-up calls to collect feedback. Storage data and fruit were presented in August in Yakima and Wenatchee in 2012 and 2014. Fruit was also available for tasting at the rootstock field day (Wapato, 8.22.12) and the Sunrise field days (8.23.12 and 8.07.13). The Quincy Phase 3 site was open to the public for field days each year with the addition of the WA 38 plots at Sunrise, Roza and Aller Road in 2014. An organic sample of WA 38 was available for tasting as part of an event at the Tilth Producers Conference, Yakima, November 2013. A WSU-CAHNRS webpage is devoted to information on WA 38: <http://cosmiccrisp.wsu.edu>.

Talks and posters:

- Presentations describing the WABP include: the ISHS Organics meeting, Leavenworth, WA (2012); Department of Agriculture and Food, Manjimup, Australia, University of Western Australia, Perth, Australia (2012); industry meeting in Santiago, Chile (2013); the INIA apple breeding facility in Chillan, Chile (2013); international AIGN nursery group meeting, Yakima (2013); IFTA annual conference, Kelowna, Canada (2014); Agrofresh Crunch Academy, Wenatchee (2014); and the 7th International Rosaceae Genomics Conference, Seattle, WA (2014).
- Examples from the WABP of DNA-informed breeding development and application were used in talks at: Wiley Research Expo, Pullman, WA (2012); FruitBreedomics 1st annual meeting, Prague, Czech Republic (2012); FruitBreedomics 3rd annual meeting, Zurich, Switzerland (2014), RosBREED 3rd and 4th annual meetings, East Lansing, MI (2012, 2013); RosBREED annual Advisory Panel meetings, San Diego, CA (2012, 2013); 2nd International

Symposium on Biotechnology of Fruit Species, Napier, New Zealand (2012); American Society for Horticultural Science (ASHS) annual conference, Miami, FL (2012); ASHS annual conference, Palm Desert, CA (2013); 6th Rosaceous Genomics Conference, San Michele, Italy (2012); 7th International Rosaceae Genomics Conference, Seattle, WA (2014); Yakima Pomology Club meeting, Pullman, WA (2012); University-Industry Consortium meeting, San Diego, CA (2012); Plant & Animal Genome Conference, San Diego, CA (2013); WSU Horticulture and Crop & Soil Science Graduate Student Tour, Wenatchee, WA (2013); International Horticulture Congress, Brisbane, Australia (2014); and in various guest lectures at WSU (2012, 2013, 2014)

- Two posters describing research associated with the WABP were presented at WSU's Academic Showcase, Pullman (2012); fruit were also available for tasting.
- Posters on DNA-informed breeding in the WABP were presented at: the ASHS annual conference, Miami, FL (2 posters, 2012); American Society for Horticultural Science annual conference, Palm Desert, CA (2013, 2 posters); ASHS annual conference, Orlando, FL (1 poster, 2014); 6th Rosaceous Genomics Conference, San Michele, Italy (2 posters, 2012); and the 7th International Rosaceae Genomics Conference, Seattle, WA (3 posters, 2014).
- March 2013 & 2014: Dr. Evans and her Wenatchee team presented 'hands-on' displays about the WSU apple breeding program to middle schoolers and the general public during the UW Gear Up Science and Engineering Festivals in Yakima.
- September 2013: Dr. Evans presented a 'hands-on' display about the WABP at the WSU3i event in Seattle which resulted in a feature on King5 news highlighting WA 38.
- October 2013 & 2014: Dr. Evans and Julia Harshman (grad student) presented the WABP to the Post-Harvest class at Wenatchee Valley College. The class also toured the Wenatchee labs and experienced sensory analysis of some selections.
- December 2012, 2013, 2014: the WABP presented its usual array of Phase 2 fruit selections for tasting at the WA Hort Show as well as having samples of both WA 2 and WA 38 available.

Good Fruit Grower articles:

- Hanrahan I, Auvil T, Evans K. (2014) WA 38 apples in storage. *Good Fruit Grower*. 65(17):78-79
- Auvil T, Hanrahan I, Musacchi S, Evans K. (2014) How to grow Cosmic Crisp. *Good Fruit Grower*. 65(12):28-30
- Evans KM, Hanrahan I, Ross C. (2014) Consumers rate WA 38. *Good Fruit Grower*. 65(11):27-29
- Barritt BH, Evans K, Brucher L. (2013) Consumer expectations of apple quality: how do 'WA 2' and 'WA 38' measure up? *Good Fruit Grower*. 64(6):44-45.
- Evans KM, Barritt BH. (2012) WSU releases WA 38 apple. *Good Fruit Grower*. 63(14):39-40
- Evans KM, Ross CF. (2012) Consumers prefer WA 2. *Good Fruit Grower*. 63(12):28.
- Hanrahan I, Auvil T, Evans KM. (2012) WA 2 updates. *Good Fruit Grower*. 63(11):20

Videos & webinars:

- Fruit testing in the WSU apple breeding program: <https://www.youtube.com/watch?v=n-oQsRAu-I>
- Cosmic Crisp™ brand WA 38 Field day September 17 and 30 2014: <https://www.youtube.com/watch?v=NrC678Khfig>
- National Association of Plant Breeders Webinar Series: <http://www.extension.org/pages/70439/making-appealing-apples:-napb-webinar#.VHa-hMk-mjM>

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 WA 38 (Cosmic Crisp™) and 2) wide-spread routine use of DNA-informed selection for fruit quality.

The demand for trees of WA 38 by Washington State growers for planting in 2017 was over 4 million trees (as taken from the drawing applications in June 2014). The first 300,000 trees available have been allocated to 24 grower entities representing large and small enterprises across the state. Rootstock and systems trials have been established and post-harvest and field evaluation have continued in the Phase 3 sites throughout this project, together producing a greater volume of data about this new variety than any other new variety at this stage in its development. Such information will go a long way towards reducing the risk taken by Washington growers in choosing to plant it.

The WABP has continued to strive for improved efficiency in all aspects of the program. The application of DNA-informed breeding is now routine with the process being efficient and streamlined from the collection of seedling leaf material through to data delivery and the subsequent identification of seedlings to be culled. The ‘three-Phase’ structure of the WABP is continually evolving, not fixed; opportunities to implement ‘half’ phases when appropriate keep the program dynamic and ahead of the game while also ensuring cost-efficiency and deliverables to the industry.

Deliverables:

1. WA 38 (Cosmic Crisp™) release plan to the Washington industry.
2. One further promising elite selection in Phase 3 production.
3. Fully routine DNA-informed breeding for fruit quality.