

FINAL PROJECT REPORT
WTFRC Project Number: AP15-102A

Project Title: Apple Scion Breeding

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Cooperators: Bruce Barritt, Professor Emeritus, WSU; 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

Other funding sources

Agency Name: WTFRC Apple Review

Amount awarded: \$107,000 (2015-2018)

Notes: “Combining fire blight resistance and horticultural quality in Washington apples” PI: Norelli. Co-PI: Evans. Synergistic project to identify sources of fire blight resistance.

Agency Name: USDA-CSREES Specialty Crops Research Initiative

Amount awarded: \$5.72M (2015-2018 with 1 more year likely)

Notes: “RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars” PI: Iezzoni. Co-PIs: Peace, Evans et al. To further develop MAB for U.S. Rosaceae crops.

Agency Name: USDA-CSREES Specialty Crops Research Initiative

Amount awarded: \$2.7M (2014-2019)

Notes: “Genome Database for Rosaceae: Empowering Specialty Crop Research through Big-Data Driven Discovery and Application in Breeding” PI: Main. Co-PIs: Evans, Peace et al. Synergistic project for application of bioinformatics to tree fruit crops.

Total Project Funding: Year 1: \$249,881 Year 2: \$266,445 Year 3: \$260,362

Budget History:

WTFRC Collaborative expenses:

| Item | 2015 | 2016 | 2017 |
|-----------------------------|--------|--------|---------------|
| Wages | 21,500 | 11,700 | 14,700 |
| Benefits | 8,600 | 7,800 | 9,800 |
| RCA Room Rental (x2) | 8,100 | 8,100 | 8,100 |
| Shipping | 0 | 0 | 0 |
| Supplies | 1,000 | 1,000 | 1,000 |
| Travel | 3,500 | 3,500 | 3,500 |
| Plot Fees | 0 | 0 | 0 |
| Total | 42,700 | 32,100 | 37,100 |

Budget 1

Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnson & Joni Cartwright
Telephone: 509 335 7667, 509 663 8181 **Email address:** carriej@wsu.edu; joni.cartwright@wsu.edu

| Item | 2015 | 2016 | 2017 |
|---------------------------------------|---------|---------|----------------|
| Salaries¹ | 59,205 | 61,573 | 64,036 |
| Benefits | 20,697 | 21,525 | 22,386 |
| Wages² | 22,680 | 23,587 | 24,530 |
| Benefits | 4,309 | 4,482 | 4,661 |
| Orchard establishment supplies | 20,000 | 20,800 | 18,060 |
| Genotyping supplies | 17,000 | 18,500 | 20,000 |
| Travel³ | 14,690 | 15,278 | 15,889 |
| Miscellaneous (virus testing) | 1,500 | 4,500 | 1,500 |
| Plot Fees | 8,800 | 8,800 | 8,000 |
| Total | 168,881 | 179,045 | 179,062 |

Footnotes:

¹Salaries for Agricultural Research Technologist (Bonnie Schonberg @ 1.0 FTE) and for 3 months for genetic screening technician (Terence Rowland @ 0.25FTE)

²Wages for time-slip labor for orchard management and trait phenotyping

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

Budget 2

Organization Name: Willow Drive **Contract Administrator:** Roger Adams
Telephone: 509 787 1555 **Email address:** roger@willowdrive.com

| Item | 2015 | 2016 | 2017 |
|------------------------------|--------|--------|---------------|
| Seedling propagation | 35,400 | 53,300 | 35,700 |
| Phase 2 & 3 trees | 2,900 | 2,000 | 8,500 |
| Total | 38,300 | 55,300 | 44,200 |

ORIGINAL OBJECTIVES

1. Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.
2. Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

SIGNIFICANT FINDINGS

1. Fifty-eight new families were made in 2015-2017 with approximately 79,800 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 43,500 seeds from 2014-2016 crosses were grown in the greenhouse.
3. Approximately 26,000 seedlings were screened with DNA markers for fruit quality; just over 15,000 were culled leaving the remaining seedlings to be transplanted to Willow Drive nursery along with another 1,100 seedlings that survived fire blight screening.

4. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. More than 10,500 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2016-2018.
5. The final count of new Phase 1 trees planted in 2015-2017 was approximately 11,080.
6. Promising selections already in Phase 2 trials (planted in 2007-2016) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Twenty-nine new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2015-2017.
8. Three new promising Phase 3 selections were planted (on Geneva 41 rootstock) at the Quincy site.
9. One new promising Phase 2 selection was top-worked onto trees at the Phase 3 Quincy site but was subsequently rejected in the 2017 BPAC meeting.
10. Fruit was harvested and evaluated through storage for new Phase 3 selections. Fruit from the two most advanced selections was available for sampling at the WA Hort Show and at the January 2018 WTFRC Apple Review.
11. A total of seven WA 38 field days were held in 2015-2017.
12. 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.
13. The Apple Cultivar Licensing Committee continued to provide input regarding the release strategy for WA 38 and future releases from the WABP.
14. WA 38 was the focus of a WA Hort Show session (12.5.16)
15. S-incompatibility alleles of most parents and selections were deduced using whole genome DNA profiling data.

RESULTS & DISCUSSION

Objective 1: Produce, through integration of traditional and DNA-informed breeding methods, promising selections and subsequently elite selections with outstanding eating quality and productivity.

Breeding program priority traits were discussed with the Breeding Program Advisory Committee (BPAC) in November 2015 and revisited at the 2016 Review dinner in the form of a clicker survey. Current target priorities were reported in the 2017 continuing report.

DNA test information was used to help design crosses for the 2015, 2016 and 2017 seasons and approximately 26,000 seedlings from this project period were screened with DNA tests for fruit quality. DNA tests used were as follows: Ma-indel (acidity, crispness, bitter pit resistance), Md-PG1_{SSR}10kdb (firmness), Md-LG1Fru-SSR (fructose content), and Md-ACS1-indel (firmness/storage). Almost 60% of these seedlings were culled in the greenhouse as they were predicted to have less than favorable fruit quality.

Approximately 9,000 seedlings were inoculated with fire blight in the greenhouse to select for resistance.

S-incompatibility alleles for the majority of WABP parents and advanced/elite selections were determined using whole-genome DNA profiles. This information is particularly useful when designing crosses to eliminate the risk of trying to make a fully incompatible cross combination.

Seedlings of 'WA 2' × 'White Angel' from the 2014 crossing season were germinated and sent to Pullman to be raised. 'White Angel' has a different source of resistance to mildew than *M. zumi*. Fei

Xiong Luo (a WSU graduate student) developed a DNA test for this 'White Angel' genetic source (Luo et al., submitted) based on genetic map information previously published by Evans and validated by the new seedling population.

Crosses were made between WA 38 and a wild apple relative to introgress fire blight resistance into the breeding program germplasm, using the three *Malus sieversii* accessions selected from the Norelli project ('Fire blight resistance and fruit quality in new Washington cultivars', CP-15-100) as having the best fruit quality. Several hundred seeds were produced from each cross combination which were screened for fire blight resistance in 2017.

All seedling tree blocks (Phase 1 plantings) are now at Columbia View orchard and the breeding program team has been working closely with Cameron Burt (TFREC farm manager) to improve management practices with a focus on improving efficiency and increasing the number of individuals that fruit by year 3. Several hundred seedling selections have been evaluated through the timespan of this project. Evaluation of stored fruit from the 2017 season was completed in the no-cost extension of early 2018.

Objective 2: Use an effective phenotypic evaluation system combined with advanced statistical analyses to identify selections with outstanding performance.

Fruit from 1,041 seedling trees were harvested and evaluated during the 2014-2017 seasons. In addition, fruit from 324 'keeper' selections were evaluated (1,166 samples in total), plus fruit from 81 Phase 2 selections and controls (3,189 samples).

All samples were routinely bar-coded at harvest and then tracked through post-harvest evaluation. Data at the end of the season were analyzed with 'Elite Advance' software, trait by trait, and top-ranking individuals were selected using a combination of these data and breeding team discussion.

The DA meter (T.R. Turoni, Italy) was tested in the 2014 season (data analysed within this project) for determining harvest maturity of Phase 2 selections. This testing confirmed that the results varied depending on skin and flesh color (white/cream/green hues). While the DA meter is a possibly useful tool for determining harvest maturity on single varieties, the diversity of the breeding program germplasm reduces its applicability. Jamie Coggins (a WSU Masters student) demonstrated that an alternative instrument, the Felix F750 Quality meter, can be used to accurately predict dry matter content and soluble solids content of Phase 2 advanced selections. Prediction models obtained from the Felix were considerably less robust for estimating acidity content or firmness. Consequently, we will have to continue to use destructive measurements to evaluate these important traits. Correlation analysis of the dry matter data with other sensory and instrumental measures is on-going, with the aim of replacing laborious destructive methods.

As a result of collaboration with Dorrie Main's NRSP10 Big Data project, all sensory data collection has been successfully transitioned into the Tablet-based FieldBook App, streamlining the last remaining part of the program that had required manual data entry.

New selections were made for both Phase 2 and Phase 3. Twenty-nine promising seedling selections were planted in Phase 2 during this project. With seven older selections (pre-2015) remaining in Phase 2, there are currently 36 advanced selections in Phase 2 or Phase 3.

Three selections were added to Phase 3 on G.41 rootstock (2015 and 2017 planting) using the staggered start system of planting initially at only one site prior to adding a second site. Fruit was harvested on

four elite selections in Phase 3 from the Quincy site and three from the Prosser site. Performance results for three elite selections were discussed at the November 2017 BPAC meeting and fruit samples were provided for tasting. The observation of a relatively large proportion of culled fruit at harvest (23% total yield) of one of these, a ‘Honeycrisp’ × ‘Cripps Pink’ selection, validated performance problems that were experienced in the 2016 season. Consequently, the decision was taken to discontinue this selection. Sufficient volumes of fruit from two other ‘Golden Delicious’-season elite selections (another ‘Honeycrisp’ × ‘Cripps Pink’ selection and a ‘Cripps Pink’ × ‘Honeycrisp’ selection) enabled samples to be provided at multiple stakeholder meetings in the 2017/18 season.

The breeding program benefits from input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the horticultural aspects of Phase 3 elite selections. An annual meeting in November each year has provided the opportunity to obtain BPAC feedback on the commercial potential of Phase 3 selections and provide input on the future relationship between the WABP and its Washington stakeholders.

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscoll, and Dale Goldy for the Quincy trial and Ray Fuller for the Phase 2 planting in Chelan. Also thanks to AgroFresh for providing 1-MCP, Stemilt for accommodating our complex needs through the storage season, and Legacy fruit packers.

WABP Publicity

Numerous fruit samples of WA 38 and Phase 3 selections were distributed to the grower industry, allied technologies industry, and other target audiences. Field days were organized to showcase WA 38 at several stages of the growing season each year. Video: <http://treefruit.wsu.edu/videos/wa-38-cosmic-crisp-field-day/>

‘Market to Market’ Iowa public TV attended and filmed a WA 38 field day in 2016.

WA 38 horticulture and commercialization was featured in a full afternoon session of the 2016 WA Hort Show (Washington Horticultural Association Show) in December. WA 38 was featured as part of the ‘Big Ideas’ celebration event on Pullman campus (9.18.15) and in a NY Times article ‘Beyond the Honeycrisp’ (11.4.15).

Talks and Posters

April 2015 – Dr. Evans presented “The WSU apple breeding program” as part of the WSU IAREC Seminar series, Prosser, WA.

July 2015 – Julia Harshman (graduate student) presented the WABP and hosted a WA 38 tasting at the National Association of Plant Breeders annual meeting in Prosser and Pullman, WA.

August 2015 – Julia Harshman presented the results of her WABP efficiency studies (in collaboration with Craig Hardner) at the American Society for Horticultural Science annual conference, New Orleans.

September 2015 – A SeedWorld interview with Dr. Evans “The challenges of apple breeding” was featured online. (<http://seedworld.com/kate-evans-associate-scientist/associate-professor-wsu-napb-annual-meeting-2015-giant-views/>)

October 2015 – Jamie Coggins (graduate student) presented WABP products and testing protocols in a middle school STEM event in Yakima.

November 2015 – Dr. Evans presented a talk ‘Breeding pome fruit in Washington State’ at the ‘Advances in field-based high-throughput phenotyping and data management’ meeting in Spokane, WA.

December 2015 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association Show in Yakima, WA. Dr. Evans also presented a talk ‘Developing and implementing new technologies for and from the WSU pome fruit breeding program’.

Jan 2016 – Korean nursery visit, TFREC, WA. (*Evans*): ‘The interaction between the WABP and the U.S. Clean Plant Network.’

Aug 2016 – National Association of Plant Breeders conference, Raleigh, NC. (*Jamie Coggins, Evans Grad student*) poster: ‘Utilizing dry matter and Near-Infrared spectroscopy for selection in the WSU apple breeding program.’

Aug 2016 – WSU CSS 512 Field crop breeding students tour of the apple breeding program (*Evans*).

Oct 2016 – Hort 509/510 seminar, WSU. (*Evans*): ‘The WSU apple breeding program’.

Oct 2016 – International Women’s Association of Yakima, Yakima, WA. (*Evans*): ‘From the U.K. to the U.S.: the science of breeding tasty new apples.’

Oct 2016 – Sarah Kostick and Jamie Coggins (*Evans grad students*) hosted visiting High School students and presented the apple breeding program with a sensory evaluation activity.

Oct 2016 – ISHS 1st International Apple Symposium, Yangling, China. (*Peace*): ‘Learning as we go: DNA-informed apple breeding at Washington State University.’

Oct 2016 – Henan Agricultural University invited seminar, Zhengzhou, China. (*Peace*): ‘From QTLs to routine DNA-informed breeding: prospects, advances, & needs ...and experiences in apple at Washington State University.’

Nov 2016 – Wageningen University & Research invited seminar, Wageningen, Netherlands. (*Peace*): ‘From QTLs to routine DNA-informed breeding: prospects, advances, & needs ...and experiences in apple at Washington State University.’

Nov 2016 – University of Maryland invited lecture, College Park, MD. (*Evans*): ‘Development and application of DNA-informed breeding in the WSU apple breeding program.’

Nov 2016 – The breeding program hosted a Fruit Evaluation class of students from Wenatchee Valley College.

Nov 2016 – 1st Tropical Genomes Conference keynote presentation, Cairns, Australia. (*Peace*): ‘DNA-informed breeding successes in temperate rosaceous crops: What can tropical crops learn?’

December 2016 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association meeting in Wenatchee, WA. Dr. Evans also presented talks entitled ‘Introducing WA 38; A new standard of product excellence’ and ‘Tree fruit breeding and selection at WSU’.

Aug 2017 – National Association of Plant Breeders conference, Davis, CA. (*Coggins, Evans grad student*) poster: ‘Utilizing dry matter and Near-Infrared spectroscopy for selection in the WSU apple breeding program.’

Aug 2017 – WSU CSS 512 Field Crop Breeding students tour of the apple breeding program (*Evans*).

Sep 2017 – Pacific Science Center WellBeing Curiosity Day, Seattle, WA (*Schonberg, Kostick, Evans team*) Apple breeding program fruit evaluation.

Sep 2017 – American Society for Horticultural Science annual conference 2017, Waikoloa, HI. (*Peace*): ‘What you see is what you can improve: Breeding utility of genome-wide haplotype mosaics’.

Sep 2017 – NRSP10: Bioinformatic and Database Resources for Specialty Crops workshop, American Society for Horticultural Science Annual Conference 2017, Waikoloa, HI. (*Peace*): ‘NRSP10 resources for translational tree fruit research’.

Sep 2017 – American Society for Horticultural Science annual conference 2017, Waikoloa, HI. (*Coggins, Evans graduate student*): ‘Utilizing visible/Near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program’.

Oct 2017 – Our Valley Our Future seminar series, Wenatchee Valley College, WA. (*Evans*): ‘Apple breeding 101’.

Nov 2017 – The breeding program hosted the Fruit Evaluation class of students from Wenatchee Valley College. (*Kostick & Coggins, Evans graduate students*)

- Nov 2017 – NC-140 multi-state project annual meeting, Wenatchee, WA. (*Kostick*): ‘Identifying sources of fire blight resistance and associated heritable loci in apple’.
- Dec 2017 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association Show in Wenatchee, WA.
- Dec 2017 - Washington State Horticultural Association Show, News flash presentations, Kennewick, WA. (*Barritt, Coggins, Kostick*): ‘Cosmic Crisp® cv WA 38 and Sunrise Magic® cv WA 2: different opportunities to satisfy consumers’, ‘Utilizing visible/near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program’, ‘Identifying sources of fire blight resistance and associated heritable loci in apple’

Scientific Papers

- Chagné D, Vanderzande S, Kirk C, Profitt N, Weskett R, Gardiner SE, Peace CP, Volz RK, Bassil NV. Validation of SNP markers for fruit quality and disease resistance loci in apple (*Malus × domestica* Borkh.) using the OpenArray® platform. *Horticulture Research* (accepted)
- Evans K, Peace C. (2017). Advances in marker-assisted breeding of apples. In: *Achieving Sustainable Cultivation of Apples*. Ed. K. Evans. Burleigh Dodds Science Publishing, Cambridge, UK. (ISBN: 978 1 78676 032 6) pp 165-194
- Hardner C, Evans KM, Brien C, Bliss F, Peace C. (2016) Genetic architecture of apple fruit quality traits following storage and implications for genetic improvement. *Tree Genetics & Genomes* 12:20. DOI 10.1007/s11295-016-0977-z
- Harshman J, Evans K, Allen H, Potts R, Flamenco J, Aldwinckle HS, Wisniewski M, Norelli JL. (2017) Fire blight resistance in wild accessions of *Malus sieversii*. *Plant Disease* 101:1738-1745.
- Harshman J, Evans K, Hardner C. (2016) Cost and accuracy of advanced breeding trial designs in apple. *Horticulture Research* 3:16008. DOI 10.1038/hortres.2016.8
- Howard NP, van de Weg E, Bedford DS, Peace CP, Vanderzande S, Clark MD, Teh SL, Cai L, Luby JJ. (2017) Elucidation of the ‘Honeycrisp’ pedigree through haplotype analysis with a multi-family integrated SNP linkage map and a large apple (*Malus × domestica*) pedigree-connected SNP data set. *Horticulture Research* 4:17003.
- Luo F, Luo F, Sandefur P, Evans K, Peace C. A DNA test for routinely predicting mildew resistance in descendants of crabapple ‘White Angel’. *Molecular Breeding* (submitted)
- Peace C. (2017) DNA-informed breeding of rosaceous crops: Promises, progress, and prospects. *Horticulture Research* 4:17006.
- Ru S, Hardner C, Carter PA, Evans K, Main D, Peace C. (2016) Modelling of genetic gain for single traits from marker-assisted seedling selection in clonally propagated crops. *Horticulture Research* 3:16015. DOI 10.1038/hortres.2016.15
- Vanderzande S, Piaskowski JL, Luo F, Edge-Garza DA, Klipfel J, Schaller A, Martin S, Peace C. (2018) Crossing the finish line: How to develop diagnostic DNA tests as breeding tools after QTL discovery. *Journal of Horticulture* 5:228.

EXECUTIVE SUMMARY

The Washington State University apple breeding program (WABP) continues to evolve to meet the demands of the Washington industry. Involvement of the industry is vital at several key parts of the program: priority trait discussion, independent evaluation for grower-friendly selections, and commercialization decisions (both selections and mechanisms).

The initial uptake of the latest release from the WABP 'WA38' has been unprecedented. Estimates of current plantings in Washington exceed 6 million trees. New elite germplasm continues to move through the cultivar development pipeline, being evaluated through the same mechanisms as 'WA 38'; this detailed evaluation is essential to provide grower confidence in new variety uptake. Almost 80,000 apple seeds were produced as a result of controlled crosses within this project cycle, with approximately 26,000 of them screened with an array of DNA tests shortly following germination. In addition, approximately 9,000 seedlings were inoculated with fire blight to determine their resistance as part of a continued effort to introgress fire blight resistance into elite germplasm.

The WABP continues to implement new technology whenever available and appropriate, and has tested two new non-destructive phenotyping methods within this project cycle. Although initial data was not promising, we hope to further evaluate these techniques possibly in collaboration with other North American apple breeding programs.

The WABP team has taken every opportunity to present data from the program at a wide range of events as well as participate in several broadcasted interviews. We will endeavor to build on current success as we move forward into the 2018 season.