FINAL PROJECT REPORT

Project Title: PNW sweet cherry breeding and genetics program

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Other funding sources

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amt. awarded: $3.4M plus equal matching Sep 2009 – Aug 2013
Notes: "A total systems approach to developing stem-free sweet cherry production, processing, and marketing system". PI: Whiting. Co-PIs include Oraguzie and Dhingra

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amt. awarded: $2.1M plus equal matching Sep 2009 – Aug 2013
Notes: "Tree Fruit GDR: Translating genomics to fruit tree agriculture". PI: Dorrie Main. Co-PIs include Oraguzie and Peace.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace, Oraguzie, and Main.

Agency name: WTFRC/OSCC
Amount awarded: $59K for 2012
Notes: "Targeting the ethylene response pathway to improve cherry quality". PI: Peace

Agency name: WTFRC/OSCC
Amount awarded: $79K for 2010-2012
Notes: Start-up funds and support for a full time technician with Oraguzie as PI

Total Project Funding: $501,046

Budget History:

Organization Name: WSU-Prosser **Contract Administrator:** Carrie Johnston
Telephone: 509 335 4564 **Email address:** carriej@wsu.edu

Item	2012	2013	2014
Salaries	24,646	25,63	46,035
Benefits	9,813	10,247	21,178
Wages	16,800	17,472	18,171
Benefits	12,953	13,471	14,010
Equipment			
Supplies	7,000	5,000	4,000
Travel	4,914	3,000	3,000
Plot Fees	7,125	4,750	4,750
Plot establishment and maintenance	40,500	40,500	40,500
Miscellaneous	600	600	600
Total	124,351	120,672	152,244

Footnotes: Salaries included 1.0 FTE for Breeding Technician in 2014 (combining CH-13-102 and this project) and 0.15 FTE for Terry Rowland (full-time genetic screening technician in Dr. Peace's Washington Tree Fruit Genotyping Lab, Pullman, WA) and 0.5 FTE for a Breeding Technician in 2012 and 2013. The other 0.5 FTE salary for Breeding Technician came from WTFRC/OSCC-funded project # CH-10-110. Wages included the equivalent of 5 temporary employees during spring and summer months. Supplies included propane, soil, stakes, chemicals for green house, growth room and field operations and other lab consumables. Elisa test was conducted on approximately 20 cultivars at bloom time for $30/tree. Land use fee was $475/acre. Plot establishment and maintenance fee was ~$4,500/acre. This was applied to ~10 acres of the breeding blocks while the cost of maintaining the other 6 acres was covered by federal grants.

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries +benefits	1,500	8,700	15,000
Wages	800	4,400	8,000
Benefits	320	1,700	3,200
RCA Room Rental			
Shipping			
Supplies		200	600
Travel	500	1,500	1,500
Miscellaneous			
Total	3,120	16,500	28,300

Footnotes: The funds are for phase 2 tree evaluation.

Organization Name: Willow Drive Nursery Inc. **Contract Administrator:** Hal Leedy
Telephone: 509 787 1555 **Email address:**
Hal@willowdrivenursery.com

Item	2012	2013	2014
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Tree propagation:			
Advanced selections	13,593	13,593	13,593
Parents	677	677	677
Breeding parents/Diversity set		9,024	
Miscellaneous			
Total	14,270	23,294	14,270

Footnotes: Tree propagation fee is $11.23 per tree. Sixty trees of 12 advanced selections will be propagated per year. Parents include market leading cultivars and checks planted alongside advanced selections. Breeding parents/diversity set includes F_1 progeny, modern cultivars and ancestors propagated for use as breeding parents, and for a workhorse pedigree set of multiple populations established in the RosBREED project for identifying and refining marker-locus-trait associations.

Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-296 5494 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries[1]	1,539	2,376	2,447
Benefits	1,154	1,782	1,835
Wages[2]	0	500	1,500
Benefits	0	50	150
Equipment			
Fees and Supplies[3]	3,604	3,354	3,354
Travel	0	0	0
Miscellaneous			
Total	6,297	8,062	9,286

Footnotes: [1]Salaries are for: 0.039 FTE (2 weeks) for technician in year 1, and 0.058 FTE (3 weeks) in years 2 and 3; to include planting, irrigation, tree training, data collection (bloom, harvest, fruit quality). OPE rate is 75%. A salary increase of 3% is factored into years 2 and 3. [2]Wages are for one part-time employee ($10/hr) to assist with tree planting, and data collection in years 2 and 3; OPE is 10%. [3]Supplies include bird netting, filters for juice analysis, lab tape, and labels. Fees include per acre research plot fee: $3,104.

Objectives

1. Develop and utilize best management practices for optimal seed germination and accelerated development of healthy seedlings in the greenhouses and for field maintenance and development of superior horticultural practices that accelerate seedling growth and development and reduce time to flowering and fruiting
2. Use elite selections from the breeding program and new external sources of genetic superiority as parents for hybridization and selfing to produce seedling populations that segregate for target traits critical to each target market group
3. Integrate genomics knowledge, marker-assisted breeding tools, and classical breeding methods into the breeding program to optimize use of resources and reduce time to release of elite selections with commercial potential
4. Develop and implement a cost-effective strategy for collaborative breeder-grower identification and evaluation of elite new selections from the breeding program
5. Identify in Phase 2 at least one elite selection from any target market class that exceeds the threshold values for the primary and secondary traits of that target market class for advancement to Phase 3

SIGNIFICANT FINDINGS AND ACHIEVEMENTS

- The breeding program has advanced to the next generation beyond named commercial cultivars, using F_1 progeny as breeding parents – allowing focus on such traits as powdery mildew resistance and self-fertility while still maintaining effort in extending the early and late market windows.
- Super-early progeny were generated by integrating early-ripening trees produced in the 1970s and 1980s into the crossing strategy. Experience with viability of the resulting early seed has been positive.
- A few of the available 300 ‘Rainier’ x ‘Cristobalina’ offspring were identified via DNA testing as suitable parents for introgression breeding for a valuable new genetic source of both extra-earliness and self-fertility. This new source for these target attributes support future generations of expanded rather than narrowing genetic diversity in the breeding program.
- Several crosses involving ‘Glacier’, ‘Kiona’, and ‘Tieton’ were performed to increase the incidence of achieving a relatively rare inbred genotype expected to confer large fruit size and very firm fruit texture as well as self-fertility.
- For artificial hybridization, hand pollination was combined with open pollination based on releasing bees on trees in bee tents. Although only the seed parent from such seedlings is known, DNA markers can later determine the source of pollen if selections arise. This strategy has potential to boost seed numbers, increase the number of F_1 seedlings, and consequently our chances to identify winners for advancement to phase 2.
- Genetic tests for self-fertility and fruit size were used for routine seedling selection. In addition, DNA evidence was used to confirm or deduce parentage for exotic pollen sources, confirming that the intended crosses were mostly successful.
- Culling of genetically inferior seedlings using DNA tests was conducted before transfer of seedling to the greenhouse, resulting in significant reductions in resource use. In 2013 and 2014, an average of 1400 seedlings were culled per year (80% of those tested). This culling provided an estimated net projected savings of ~$30K, allowing resource reallocation to more promising breeding operations rather than raising and evaluating genetically inferior seedlings.

- Tall (3- to 4-foot) saplings are routinely produced in the greenhouse, reducing generation times such that fruit are often now produced in the third year following field planting.
- Introgressing powdery mildew (PM) and bacterial canker (BC) resistance into elite selections was a major emphasis in recent years of the breeding program. Progenies of 'AA', 'BB', 'CC', 'DD', 'EE', the 'MIM' series accessions, and 'Regina' that have combined PM and BC resistances and good fruit quality were crossed to 'Sweetheart' and also intercrossed to increase resistance durability and enrich resistance alleles in the breeding genepool.
- Based on input from strategic BPAC members and continued observation of horticultural performance, fruit production, and quality, several previous Phase 2 selections were identified as flawed and subsequently were discontinued.
- 45 advanced selections are currently in the program in Phase 2, of which 19 are planted in Phase 2 trials at WSU-IAREC, Prosser, WA and OSU-MCAREC, Hood River, OR; late-ripening selections were planted at a grower trial in N Wenatchee, while early-ripening selections were planted at an early site in Pasco. The other 26 more recently identified selections are undergoing propagation in the nursery and will be planted in Phase 2 trials in the coming years.
- One Phase 2 advanced selection, Roza 2, was selected for the first Phase 3 trials. Roza 2 is in the EM market class (early, self-fertile, and mahogany) and is larger and firmer with better flavor than 'Chelan', the standard cultivar of this market class. Roza 2 trees were planted in grower trials in Orondo (WA), Pasco (WA) and the Dalles (OR). More Roza 2 trees will be planted next year at grower orchards in Buena (WA) and Wenatchee (WA).
- Roza 2 is virus free and budwood was sent from the National Clean Plant Network (NCPN) program in Prosser to Willow Drive Nursery for propagation in 2014. More certified budwood will be sent to other licensed nurseries next year.

RESULTS AND DISCUSSION

1. Develop and utilize best management practices

The PNWSCBP has continued to achieve more than 60% seed germination and more than 95% seedling survival in Phase 1 involving own-rooted trees. In addition, transition of seedlings from the growth room to the greenhouse is now limited to those seedlings that show DNA-based genetic potential, thus saving an estimated ~$30K per year on unnecessary potting soil, labor, and greenhouse space. These savings are enabled by DNA testing in the Pullman-based Washington Tree Fruit Genotyping Lab and culling inferior seedlings when the seedlings are ~2 months old in the growth room. Previously, DNA tests were performed on 9-10 month-old seedlings immediately prior to field planting, a stage involving slightly less resource savings. Another significant achievement is production of 3- to 4- foot tall trees in less than one year prior to field planting. This rapid growth helps to reduce generation time as these trees start flowering two years after field establishment and become fully productive in their third year, saving at least a year from previous practices.

All trees in phases 1 and 2 are now trained to the *central leader* architecture. The written protocol for pruning and training has been incorporated in the updated "Best Management Practices" handbook which is available upon request from PNWSCBP staff. Other advances in management practices are detailed below.

a. Sample tracking system

Each tree in the seedling blocks is tagged with a unique plastic bar-code ribbon identifying the location of the tree. During harvest, the barcode is scanned and reproduced as a stick-on label that is

fixed to the collection bag. Upon entering the fruit evaluation laboratory, each sample label is immediately logged into a universal data collection file via bar code scan, to provide a record of collection which includes the tree location and date. A reproduced label accompanies each individual fruit sample as it rotates through the evaluation stations, minimizing and/or eliminating hand-written errors. Evaluation results are entered into the data collection file by electronically scanning the unique label, which automatically locates the correct line (sample location/collection date) for data entry, eliminating mis-match errors.

b. Sample collection for marker-assisted seedling selection

Leaf samples are collected from nascent seedlings still in the yellow cone-tainers in the growth room. Earlier collection and analysis limits transplantation of seedlings to those showing genetic potential. Seedlings are identified only by parentage until leaf samples are taken, at which time each sampled individual is collared with a barcoded tag identifying year of seed harvest, seed parents, stratification bag number (for cross reference, lending additional assurance for accurate heritage assignment), and individual seedling number. Barcodes are scanned directly into a spreadsheet matrix duplicating the configuration of the leaf sample collection plates so no handwritten collection sheets are generated. Spreadsheets for each collection plate are then sent in electronic format to the lab performing the DNA tests, again eliminating hand-written error.

c. Greenhouse seedling transplanting

Seedlings showing genetic promise are transplanted to smaller pots (2 gallons) at transplant time, saving money on potting soil, labor, and greenhouse space. Previously, all seedlings (both favorable and inferior determined by genetic tests) were transplanted into 5 or 10 gallon pots before genetic tests were performed.

d. Bird control

In addition to bird netting in the Phase 1 seedling block, selected Phase 1 trees, particularly early ripening selections, are enclosed in portable bird cages constructed by the Breeding staff. To reduce the risk of bird damage, these cages are transferred from early- to mid- to late-ripening selections as the season progresses. Further, a Bird-gard unit, emitting eight different distress calls, mounted in the seedling block provides additional control.

2. Produce seedling populations that segregate for target traits critical to each target market group

Crossing decisions were guided by the breeding effort assigned to each of six target market classes (Table 1).

Table 1: Assignment of crossing effort in 2014

Market class	Breeding effort (%)	Target no. of seeds
EM	30	1200
EB	10	400
MM	10	400
Mech-M	10	400
LB	10	400
LM	30	1200
Total	**100**	**4000**

EM = early, self-fertile, mahogany' EB = early, self-fertile, blush; MM = mid-season, self-fertile, mahogany; Mech-M = early, mid-, or late-ripening, self-fertile, mahogany, suitable for mechanical harvest; LB = late, self-fertile, blush; LM = late, self-fertile, mahogany

The PNWSCBP has moved on from using standard and/or commercial cultivars as breeding parents to the use of advanced selections and seedlings arising from the breeding program with unique

attributes and/or DNA-based alleles of interest such as powdery mildew resistance and new sources of self-fertility, earliness, and lateness. This shift is further facilitated by propagating potential parents on Gisela 6® rootstock, as fruit set on own-rooted trees is poor due to erratic flowering.

During the last three years, numbered selections from breeding efforts in the 1970s and 1980s, which are useful sources of earliness and lateness with fruit size above 10 g and firmness >275 g/mm, were DNA-tested and intercrossed or mated with early-ripening advanced selections to generate progeny that are expected to include super earliness. Seed germination tests in our lab suggest that such progeny produce viable seed. Previously, due to embryo abortion and poor germination, breeding for early ripening was limited to crossing an early-ripening variety to a later-ripening variety. For example, there is usually no pollination and fruit set when 'Chelan', the earliest commercial cultivar in the Pacific Northwest, is used as a seed parent so it is mainly used as a pollen source for earliness.

A new type of self-fertility differing genetically from S4' (the typical self-fertility source of 'Stella' and present in 'Lapins', 'Sweetheart', 'Benton', and 'Selah') in combination with earliness was efficiently introgressed into breeding germplasm by using directly as parents several 'Rainer' x 'Cristobalina' seedlings. This introgression approach enriches the early-ripening genepool for new cultivar development and diversifies the germplasm base. The 'Cristobalina' grandparent of the new breeding families is a Spanish landrace cultivar that itself is early and small-fruited with a unique source of self-fertility that, unlike S4', is non-gametophytic and resides in the genome at a different locus to the *S* locus. Diversifying our germplasm base minimizes a future of reduced genetic possibilities for the PNWSCBP from constant selection for a single allele and increases the chances of capturing heterotic effects through development of offspring that out-perform their parents. Finally, we selfed self-fertile 'Glacier' and 'Kiona' (both with large fruit but poor firmness) and, in addition, mated these with 'Tieton,' to encourage development of cultivars that are self-fertile, early-ripening, and with large, super-firm fruit expected from a double dose of allele "237" of the marker BPPCT034. Allele "237" is rare in our germplasm. 'Tieton', 'Glacier', 'Kiona', and two other cultivar parents possess this allele in combination with other alleles but the genotype 237: 237 (double dose) is lacking in breeding individuals.

New sources of late ripening are in short supply. The PNWSCBP has only two Phase 2 selections that are as late ripening as Sweetheart. This is because there is no other available cultivar as late as 'Sweetheart' and recently we discovered using DNA markers that crosses made in the program in 2004 thought to be 'Sweetheart' x 'Regina' to target lateness were actually 'Lapins' x 'Regina'. This outcome could have been possible either through incorrect labeling of the mother tree or seedlings. However, many crosses in the past few years have involved 'Sweetheart' as a parent crossed with another late cultivar, although resulting seedlings are yet to fruit. Also, high breeding values for both lateness and firmness were identified (using high-resolution RosBREED data) for one of the numbered selections from the 1970s and several mature seedlings of 'Ambrunes', and these individuals were used as parents. 'Ambrunes' is another Spanish landrace cultivar that is late, firm, small fruited, and has low pedicel fruit retention force. Intermating the late selections and crossing with cultivars such as 'Sweetheart' is expected to enrich breeding populations with new sources of lateness superior to 'Sweetheart'. Further, crosses were made to combine powdery mildew resistance with good fruit quality using mature seedlings of BB, CC, DD, AA, 'Moreau', and the Mildew-Immune Mazzard series accessions (of the USDA's National Clonal Germplasm Repository, Davis, CA) crossed with progenies of 'Ambrunes', 'Regina', 'Rainier', 'Sweetheart', and others known to be late-ripening and having high firmness and large fruit.

A major challenge for controlled crosses continues to be the moderate seed output because of adverse weather conditions during the pollination season. In the last season, a combination of wind machine, irrigation water, and propane burners were not able to sufficiently mitigate the frost damage to

thousands of hand-pollinated flowers. The PNWSCBP is currently exploring other options to encourage a high level of successful pollination and fruit set. In the meantime, use of open-pollinated seed is a means of boosting seedling numbers, although such seedlings are DNA-tested just like controlled-cross seedlings to ensure that planted seedlings are enriched for desirable alleles. Last spring, we released bees onto trees in bee netting and obtained fruit set considerably higher than in hand-pollinated trees. This approach appears less complex than open pollination without bee management because it will be relatively easier to deduce the pollen parentage of seedlings from a limited number of parents s well as pollen sources available to net-contained bees, using DNA markers.

3. **Integrate genomics knowledge, marker-assisted breeding tools, and classical breeding methods into the breeding program**

DNA tests for self-fertility and fruit size are routinely used for both parent selection and for culling inferior seedlings before field planting. We also use the DNA tests on advanced selections to establish genetic identity and for genetic potential characterization. As discussed in the previous activity, DNA-based information was used to re-assign parentage to seedlings from crosses made in 2004 that were thought to be 'Sweetheart' x 'Regina'.

Of 300 seedlings from a 2006 cross between 'Rainier' and 'Cristobalina', using a new DNA test for the unique source of self-fertility from 'Cristobalina' as well as the standard DNA test for fruit size, only 16 individuals were detected to be suitable for use in introgression breeding. Introgression breeding is the incorporation of one or more desirable alleles from a non-elite/non-adapted exotic source into an elite genetic background via at least two generations of crossing. The 16 seedlings had favorable genotypes for both fruit size and self-fertility, which was corroborated by fruit size performance data. Numbered selections from breeding efforts in the 1970s and 1980s that are sources of earliness and lateness were also DNA-tested for fruit size and self-fertility and used in controlled crosses to target the genetics for early-ripening fruiting.

We are discovering new marker-locus-trait associations (addressed in the WTFRC project entitled "New genomic regions controlling production and fruit disorder traits") and converting them into new DNA tests (addressed in the project entitled "After RosBREED: Developing and deploying new sweet cherry DNA tests") to extend the efficiencies of marker-assisted breeding to additional valuable attributes.

4. **Develop and implement a cost-effective strategy for collaborative breeder-grower identification and evaluation of elite selections from the breeding program**

To date, 50 advanced selections have been identified (Tables 2, 3, and 4) and 19 of them have been planted in Phase 2 trials at WSU-IAREC experimental station in Prosser, WA and the Oregon State University (OSU) Mid-Columbia Agriculture Research and Extension Center (MCAREC) in Hood River, OR. Five trees of each advanced selection and one tree of a standard cultivar for comparison are planted in Phase 2 trials. There are also two grower trials in north Wenatchee (providing a late-season trial site) and at Sagemoor Farms in Pasco (providing an early site). Late-ripening selections, including Roza 5 and Roza 6, were planted at the late-season site in north Wenatchee while the Pasco site has plantings of early-ripening selections including Roza 2 and Roza 25. Input from BPAC members, including Dena Ybarra, Dave Allan, and Jeff Cleveringa, was very helpful in identifying flaws in some advanced selections and in pulling them out accordingly. For example, due to excessive heat last season, FR001T073, a late-ripening selection, had poor firmness and was discontinued. This was also the case with an early-ripening selection, FR001T005, which had more than 50% stylar splitting although the fruit size was greater than 10 g and firmness more than 300 g/mm. In addition, two advanced selections, FR049T083 and FR013T004, with a combination of excessive splitting and blind wood, were pulled out. FR009T033 and Roza 25 were tentatively selected two years ago

because of earliness, but 2014 records indicated that FR009T033 is unworthy of advancement to Phase 2 based on the combination of excessive splitting, small fruit size, low firmness, and high surface pitting incidence.

Table 2: Current Phase 2 advanced selections of the PNWSCBP and locations they are planted.

Selection label	P2 Name	Year planted	Harvest date	Target market class	Location planted
FR009T033*		2013	10-Jun	EM	WSU, OSU, Pasco
FR009T089	Roza 25	2013	13-Jun	EM	WSU, OSU, Pasco
FR009T049	Roza 3	2014	17-Jun	EM	WSU, OSU, Pasco
FR001T007	Roza 2	2012	18-Jun	EM	WSU, OSU, Pasco
FR001T002	Roza 1	2014	18-22 Jun	EM	WSU, OSU, Pasco
FR001T005*		2013	19 June	EM	WSU
FR010T051	Roza 15	2012	23-Jun	EM-MM	WSU, OSU
FR011T059	Roza 16	2012	27-Jun	EB	WSU, OSU
FR044T083	Roza 17	2014	27-Jun	MM	WSU, OSU,
FR001T036	Roza 8	2012	1-Jul	MM	WSU, OSU
FR049T125	Roza 18	2014	3-Jul	MM	WSU, OSU,
FR049T083*		2013	3-Jul	MM	WSU, OSU
FR002T063	Roza 10	2012	3-Jul	LM	WSU, OSU
FR004T029	Roza 12	2013	5-Jul	LM	WSU, OSU,
FR006T059	Roza 13	2012	6-Jul	LM	WSU, OSU
FR001T074	Roza 6	2011/2012	6-Jul	LM	WSU, OSU, Wen
FR006T063	Roza 14	2012	7-Jul	LM	WSU, OSU
FR002T030	Roza 9	2012	7-Jul	LB	WSU, OSU
FR002T074	Roza 11	2014	7-Jul	LB	WSU, OSU,
FR013T004*		2013	12-Jul	LM	WSU, OSU, Wen
FR009T037	Roza 7	2013	12-Jul	LB	WSU, OSU, Wen
FR001T004	Roza 4	2014	14-18 Jul	Mech-M	WSU, OSU, Wen
FR001T070	Roza 5	2013	18-Jul	LB	WSU, OSU, Wen

WSU = Washington State University-IAREC, Prosser, WA; OSU = Oregon State University, MCAREC, Hood River, OR; Wen = Wenatchee (north); * = individuals to be discontinued. FR001T005 has already been pulled out.

Table 3: Attributes of 11 selections made in 2013 from Phase 1 trials, targeted for advancement to Phase 2. Standard cultivars are included for comparison; attributes for selections that were higher than the standards in 2014 Phase 1 trials are shown in **bold**.

Selection label	P2 name	Harvest date	Market class	Fruit wt (g)	Firmness (g/mm)	PFRF (kg)	SSC (°Brix)	TA (%)
Chelan		Jun 13	EM	9.3	266	0.75	21.5	na
FR014T012	R 19	Jun 5	EM	9.2	**393**	**1.05**	22	0.46
FR036T035	R 21	Jun 19	EB	**18.2**	**315**	**0.80**	**23**	0.51
FR035T087	R 20	Jun 20	EB	13	**308**	0.64	16	0.77
Bing		Jun 22	MM	10.1	273	0.74	17.3	0.66
FR050T105	R 27	Jun 19	MM	10.4	271	0.47	**19**	**0.72**
FR051T113	R 28	Jun 21	MM	**12**	**356**	0.73	17.5	**0.93**
FR046T105	R 26	Jun 23	MM	**11**	**303**	**1.05**	**19**	**0.90**
Rainier		Jul 2	LB	13.5	331	na	15.4	na
FR044T074	R 24	Jun 20	LB	**14.7**	**353**	0.73	**17**	0.71
FR041T014	R 23	Jun 27	LB	11	320	0.70	**19**	0.73
Sweetheart		Jul 13	LM	10.3	301	0.83	25	0.64
FR052T095	R 29	Jun 24	LM	**16.7**	**370**	0.59	18	0.41
FR040T090	R 22	Jun 27	LM	**16**	**340**	0.59	18	**0.80**
FR044T083	R 17	Jul 5	LM	**12**	300	0.36	18	**0.77**

R= Roza; PFRF = pedicel fruit retention force; SSC = soluble solids content; TA= titratable acidity; na = information not available

Table 4: Attributes of 15 selections made in 2014 from Phase 1 trials, targeted for advancement to Phase 2.

Selection label	Harvest date	Market class	Fruit wt (g)	Firmness (g/mm)	PFRF (Kg)	SSC (% Brix)	TA (%)
FR052T101	4-Jun	EB	11.65	333.09	1.089	16.40	0.78
FR034T022	5-Jun	EB	13.09	295.82	0.725	17.40	0.52
FR067T131	5-Jun	EB	9.52	377.35	0.989	18.90	0.76
FR026T074	9-Jun	EB	12.69	338.53	1.028	18.20	0.79
FR022T004	17-Jun	EM	13.79	394.58	0.814	18.30	0.93
FR051T094	24-Jun	EB	12.67	392.52	1.110	22.00	1.18
FR050T058	30-Jun	MM	17.40	299.62	1.139	20.70	1.06
FR059T044	30-Jun	MM	11.45	298.90	0.450	19.20	0.67
FR051T113	30-Jun	LB	13.83	327.52	0.343	18.80	1.02
FR044T010	4-Jul	Mech-M	14.97	330.43	0.259	18.00	0.40
FR045T076	4-Jul	LB	13.65	305.45	0.554	21.00	0.82
FR050T074	7-Jul	LB	16.61	340.10	0.601	18.40	1.07
FR057T087	9-Jul	Mech-M	13.88	290.17	0.311	21.90	0.95
FR061T124	9-Jul	LM	14.36	309.61	0.668	19.40	0.80
FR072T074	10-Jul	LM	13.62	300.76	0.398	19.20	0.87

Identify in Phase 2 at least one elite selection from any target market class that exceeds the threshold values for the primary and secondary traits of the target market class for advancement to Phase 3.

One elite selection, Roza 2, has been identified and advanced to Phase 3. This selection is self-fruitful and belongs to the EM market class. The harvest timing is similar to 'Chelan' but it is larger, firmer, and more flavorful than 'Chelan'. Trees of this selection propagated on Gisela 6® were planted in three grower sites at Orondo (WA), Pasco (WA) and The Dalles (OR). More trees of Roza 2 will be planted in 2015 in grower sites at Buena (WA) and Mattawa (WA). Virus-clean budwood was sent to Willow Drive Nursery to build an inventory of virus-clean trees while more budwood will be sent to other licensed nurseries next year. At the MCAREC in Hood River, one selection had a sufficient volume of fruit to evaluate fruit quality (Table 5). A range of 4-5 d in bloom dates was observed for all flowering (i.e., 2nd leaf and older) genotypes in Phase 2 at Hood River (Fig. 1).

Table 5: 2014 fruit quality of Roza 8 at harvest (from the MCAREC, Hood River Phase 2 trial) and following 21 d of cold storage.

Genotype	Avg. yield	FF	Fruit dia.	Fruit wt.	SSC	TA	Pedicel ret. force	Skin color	Cracking	Pitting	Bruised
	lb/tree	g/mm	mm	g	%	%	g	ctifl	%	Index‡	%
	Harvest (26-June)										
FR1T36	6.2	279 a	29.8 a	11.8	19.9 a	0.73	735.5	5.4	36	na	na
Bing*	na	240 b	27.5 b	na	17 b	0.7	na	5	na	na	na
	Postharvest (+21 d stored at 32° F)										
FR1T36		346.9	30.5	11.9	18.3	0.6	694.6	5.2	na	2.3	17.6

‡ Pitting was imposed according to protocol developed by T.Auvil et al. Data are expressed as a pitting index, where 1=no pitting, 2=superficial, slight pitting, 3=moderate pitting, 4=severe pitting; n=50 per rep

* Bing used as commercial standard given that FR1T36 falls into the mid-mahogany market class

Bing fruit were from 10-year-old trees at MCAREC, commercially harvested on 26-June

Means are averages of 5 single tree replicates. Different letters within columns signify significant differences.

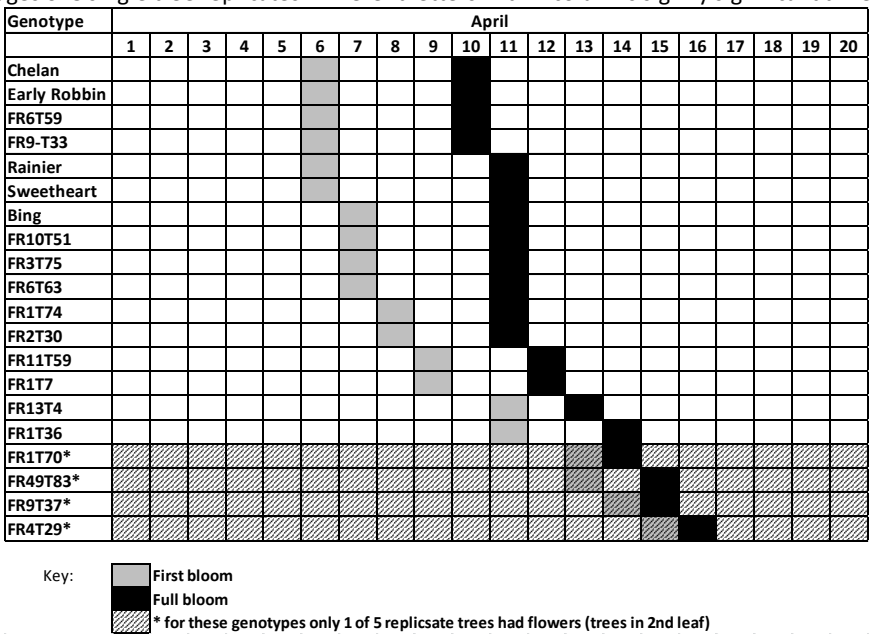

Figure 1. First and full bloom dates of Phase 2 selections with flowers Spring 2014 at the MCAREC, Hood River trial.

EXECUTIVE SUMMARY

The PNWSCBP is now a full-fledged breeding program with functional on-station and replicated multi-location on-farm trials underpinned by best horticultural practices. In Phase one (P1), DNA information is used to select parents and design crosses with a greater likelihood of carrying superior attributes. Selection emphasizes early and late ripening to extend the harvest window as well as reduction of production constraints such as disease susceptibility, pitting susceptibility and rain-induced cracking incidence. Individuals that out-perform the current leading cultivars in fruit quality traits in their target market class are selected and moved on to phase 2 (P2). P2 currently has 45 selections with 19 of these planted in P2 performance trials at WSU-IAREC, Prosser and OSU-MCAREC, Hood River. Early- and late-ripening selections are also planted in grower co-operator trials in Pasco (Sagemoor Farms) and Wenatchee, respectively. The other 26 advanced selections are in the nursery awaiting distribution and planting in the coming years. In P3, one elite selection, Roza 2, has been planted in three grower sites: in Orondo (WA), Pasco (WA) and The Dalles (OR). Trees of this selection will be planted next year in more grower sites at Buena (WA) and Mattawa (WA). This selection is early-ripening (similar to Chelan), self-fertile, and has firmer, larger, and more flavorful fruit than Chelan. Virus-clean budwood from this selection has been sent to Willow Drive Nursery for propagation while more budwood will be sent to other licensed nurseries for propagation next year.

Future plans for the PNWSCBP include planting and evaluating more P 2 selections to identifying outstanding individuals in at least one target market class for advancement to P 3. In addition, more trees of Roza 2 will be planted in more early sites in P 3 and extensive evaluation will be carried out to determine stability of performance and to inform decision on whether to release or not. We will also be collaborating with Washington Department of Agriculture (WSDA) and the Clean Plant Network (CPN) program in Prosser to develop a plan to facilitate developing virus free plants right from P 1 to avoid the laborious, time consuming and expensive process of eliminating viruses from promising selections towards the end of P 2.