

FINAL PROJECT REPORT
WTFRC Project Number: PR-19-108

YEAR: 3 of 3

Project Title: Pear Rootstock Breeding

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Cooperators: Joseph Postman (USDA-ARS Corvallis, OR), Nahla Bassil (USDA-ARS Corvallis, OR), Sara Montanari (Plant and Food Research, New Zealand), Stefano Musacchi (WSU-TFREC),

Total Project Request: **Year 1:** \$104,731 **Year 2:** \$108,371 **Year 3:** \$108,541

Other Funding Sources

Agency Name: Northwest Nursery Improvement Institute

Amount Awarded: \$9,070 (2020 – 2021)

Notes: “Assessing effects of chemical and hormonal treatments on germination of hybrid *Pyrus* rootstock seeds” (PI: Teh; Co-PI: Evans)

Synergistic project to test effects of chemicals and hormones in improving germination of hybrid *Pyrus* seeds.

Agency Name: Fresh & Processed Pear Committee Research

Amount Awarded: \$120,000 (2019 – 2021)

Notes: “Evaluating dwarfing capacity of 65 diverse pear germplasm accessions” (PI: Dhingra; Co-PI: Evans)

Synergistic project to evaluate the dwarfing capacity of diverse germplasm to be used as parental material in pear rootstock breeding.

Agency Name: Fresh & Processed Pear Committee Research

Amount Awarded: \$34,133 (2017 – 2019)

Notes: “Greenhouse screening of 49 dwarf rootstock candidates” (PI: Dhingra; Co-PI: Evans)

Synergistic project to evaluate the dwarfing potential of aneuploid pear rootstock seedlings.

Agency Name: Program Royalties

Amount Awarded: Ph.D. Research Assistantship (2019-2023) Zara York

Notes: “Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock breeding populations.” (PI: Evans)

WTFRC Collaborative Expenses: None

Budget

Organization Name: WSU-TFREC

Contact Administrator: Anastasia (Stacy) Mondy

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Item	2019	2020	2021
Salaries ¹	\$52,358	\$54,452	\$56,630
Benefits ¹	\$17,011	\$17,691	\$18,399
Wages ²	\$6,240	\$6,490	\$6,750
Benefits ²	\$4,412	\$4,588	\$4,772
Equipment & Supplies (TFREC)	\$19,600	\$19,200	\$15,200
Travel ³	\$3,190	\$3,190	\$3,190
Plot Fees	\$1,920	\$2,760	\$3,600
Total	\$104,731	\$108,371	\$108,541

¹Salaries for postdoctoral research associate (Evans lab) who is the point person for pear rootstock;

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

³In-state travel between TFREC and orchards for orchard management and trait phenotyping.

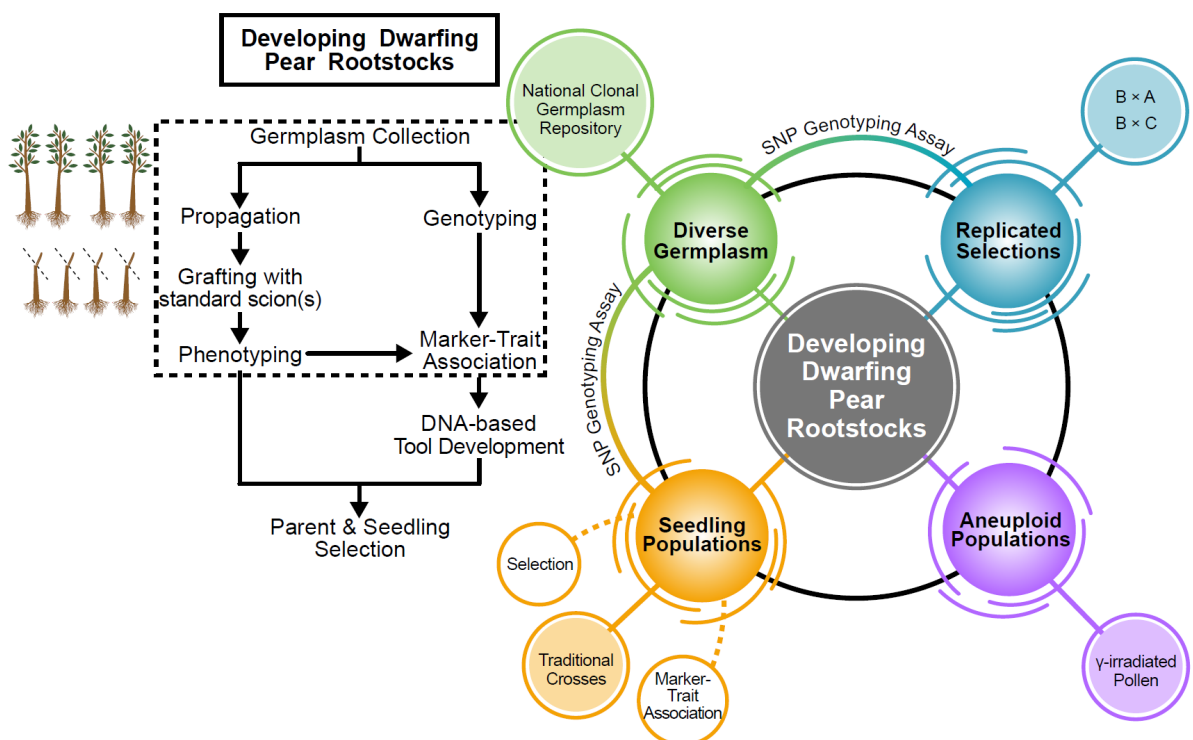
RECAP OF THE ORIGINAL OBJECTIVES

1. Develop seedling populations to produce new rootstocks
2. Validate published markers for parent and seedling selection
3. Conduct marker-trait association for dwarfing-related traits in seedling populations
4. Expand the pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

This project aims to build on a previous project (PI: Evans “Pear rootstock breeding”; PR-15-105) to develop a long-term, dedicated pear rootstock breeding program at the Tree Fruit Research and Extension Center, Wenatchee. Diverse germplasm that was previously collected from USDA-ARS, Corvallis is being used as crossing parents. New germplasm will be produced using traditional breeding of crossing and selection. DNA genotyping/sequencing using previously developed pear genomic resources (PI: Neale “Development of marker-based breeding technologies”; PR-14-111) is currently underway. In the upcoming year, genetic maps will be built using these DNA sequences. These genetic maps can then be associated with phenotypic data of rootstock-related traits to identify genomic regions associated for dwarfing (and precocity, if available), which can be developed into a DNA-based tool to enable selection of dwarfing individuals (parents or seedlings). However, this DNA-based tool development is beyond the timeframe of this project.

SIGNIFICANT FINDINGS

- ~2,000 seedlings (from 2016, 2017 and 2019 crosses) are being maintained at the WSU Columbia View orchard. These seedlings were budded with d’Anjou. Evaluation for dwarfing potential is ongoing. Up to 3 years of rootstock and scion trait data were collected.
- Ten preliminary precocious seedlings with medium to high early dwarfing effect were identified and micropropagated.
- Breeding parents were tested with published DNA markers reported to be linked with dwarf or dwarfing traits in apple and/or pear. None of the reported dwarf or dwarfing alleles (i.e., genetic copies) were present in the *Pyrus* rootstock parent set.
- Four high-density genetic maps for two seedling populations were constructed.
- A preliminary locus (i.e., genetic determinant) for dwarfing/vigor was mapped on chromosome 15 in one seedling population.
- ~45 replicated B × A and B × C selections were phenotyped for vigor-related traits, which were highly correlated. Trees are just starting to fruit with six accessions bearing fruit in fall 2021.



† Replicated aneuploid populations will be transferred from the Dhingra lab to the Waite USDA lab in 2022.

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks.

Accomplishments highlighted within the dotted box include (a) expansion of existing seedling populations, (b) propagation of rootstock seedlings with ‘d’Anjou’, (c) collection of scion and rootstock phenotypic data, (d) DNA genotyping/sequencing, (e) construction of genetic maps, and (f) marker-trait association to identify DNA regions associated with dwarfing potential.

RESULTS AND DISCUSSION

Objective 1: Develop seedling populations to produce new rootstocks

Seedling populations were generated for future selection of promising rootstocks with dwarfing potential. All seedlings were budded with d’Anjou during the fall that they were transplanted at the WSU Columbia View orchard. Vigor/dwarfing potential of rootstock seedlings and scion traits were collected annually, as shown in **Table 1**. Seedlings will be maintained for further evaluation of rootstock and scion traits, as a measure of vigor and precocity (as relevant).

Cross year	Number of seedlings	Data collection	
		Rootstock traits	Scion (d’Anjou) traits
2016	~600	Branch angle (2019) Presence of spine (2019) Trunk diameter (2020-2022)	Branch angle (2020-2022) Floral bud count (2021) Internode length (2020-2022) Scion growth (2020-2022) Trunk diameter (2020-2022)
2017	~320	Branch angle (2020) Presence of spine (2020)	Scion growth (2022) Trunk diameter (2022)

2019	~1,000	Branch angle (2022) Presence of spine (2022)	
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Table 1: Data collection of various rootstock seedling and scion (d’Anjou) traits for breeding and selection.

Improvements were made to the seedling protocol in 2019 to reduce the time that seedlings spent in the greenhouse, where typically they were subjected to high disease/fungicide pressure. The 2019 seedlings were moved from the greenhouse (after germination in spring 2020) to the WSU-TFREC hoop house in summer 2020 through spring 2021 (included overwintering). They were irrigated with auto-sprinklers, protected with nets (10% shade factor), and straw-mulched for overwintering. Hoop house space, irrigation set-up and protective nets were kindly provided by Dr. Lee Kalcsits. These seedlings established significantly better than previous years seedlings when transplanted at the WSU Columbia View orchard in spring 2021.

In 2021, ten preliminary precocious seedlings were identified and micropropagated.

Seedling populations are being leveraged through ongoing collaboration with funds via Dr. Sindhuja Sankaran (WSU Department of Biological Systems Engineering) to phenotype canopy architecture using remote sensing technologies. In fall 2021, canopy structures of approximately 450 seedlings were captured, phenotyped and extracted using remote sensing tools (LiDAR and RGB). This added layer of phenotypic data will likely enable more efficient, reliable and accurate of phenotyping canopy volume and dwarfing for our future populations.

In addition, these populations were leveraged through collaboration with funds via Dr. Lee Kalcsits to understand scion-rootstock water relation, which can be indicative of vigor/dwarfing. Plant water relation is estimated through carbon isotope composition analysis. In several studies of apple rootstocks (by Kalcsits program), positive values of carbon isotope composition were correlated with lower water availability/relation, an indicator of dwarfing. In fall 2021, leaves from our pear seedling populations were collected and dried for carbon isotope composition analysis. This biochemical/physiological information may provide additional confirmation of our existing and future vigor/dwarfing data.

Objective 2: Validate published markers for parent and seedling selection

Several DNA-based markers were reported to be linked to dwarf (e.g., *PcDw*) or dwarfing (e.g., *Dw1*, *Dw2*) traits. These markers need to be tested on our pear breeding parents to determine the allelic (i.e., genetic copy) presence and polymorphism/differences (i.e., genetic copies are different among parents). If a known dwarf or dwarfing allele is present in our breeding parents, future work would assess if phenotypic differences in the seedling populations are associated with the presence/absence of the dwarf or dwarfing allele.

Fresh young leaves from the rootstock parent germplasm were collected. DNA extraction of the parent set was carried out at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for DNA genotyping/sequencing.

DNAs of five breeding parents and one apple Bud9 reference were tested with genetic markers associated with dwarf (pear – *PcDw* locus; **two markers**), dwarfing (apple – *Dw1*, *Dw2*; **four markers**), and dwarfing (apple – *Rb1*, *Rb2*, *Rb3*; **three markers**) to determine if there are differences in the genetic copies (i.e., alleles) of these parents.

Preliminary triplicate analysis of two **dwarf markers** (pear – *PcDw* locus) showed marginal differences in the alleles between dwarf control ‘Le Nain Vert’ and our tested breeding parents.

Subsequently, high-resolution capillary electrophoresis was performed to quantify/validate the marginal differences. Results showed that none of the tested breeding parents contains the dwarf alleles of ‘Le Nain Vert’. One hybrid breeding parent has an allele in common with ‘Le Nain Vert’, but the allele is not associated with dwarf.

Preliminary triplicate analysis of seven **dwarfing markers** (apple – *Dw1*, *Dw2*, *Rb1*, *Rb2*, *Rb3*) showed clear differences of alleles between dwarfing control ‘Bud9’ and our tested breeding parents. Subsequently, high-resolution capillary electrophoresis was performed to quantify/validate the differences. Results showed that none of the tested breeding parents contains the dwarfing alleles of ‘Bud9’. One breeding parent has an allele (from *Dw1* marker) in common with ‘Bud9’; however, the allele is not associated with dwarfing.

None of these published markers (dwarf and dwarfing) is useful for pre-selecting rootstocks in our current parent set.

Objective 3: Conduct marker-trait association for dwarfing-related traits in seedling populations

Fresh young leaves from over 600 seedlings (of the four *Pyrus* seedlings populations) were collected. DNA extraction was conducted at WSU Pullman – Dhingra lab. The DNA quality and quantity were verified to meet the threshold needed for DNA genotyping/sequencing. Of the > 600 seedling DNAs, 190 were submitted in 2019 for high-resolution pear genotyping/sequencing array, which was a pear genomic tool previously developed by Dr. David Neale’s group (“Development of marker-based breeding technologies”; PR-14-111). Continued close collaboration within the U.S. and international pear genomics community facilitated cost efficiencies in genotyping analysis.

Once the 190 seedlings (of two populations) were sequenced with the genotyping array, four high-density genetic maps were constructed. In combination with phenotypic data collected in *Objective 1*, these maps were used to identify genetic determinants associated with dwarfing and/or vigor-related traits – an analysis termed marker-trait association.

Based on 2020 phenotypic data, preliminary analysis revealed a dwarfing/vigor locus that was mapped on chromosome 15. This locus was identified in one population but could not yet be validated in the other. This preliminary discovery needs additional years of phenotypic data to confirm the statistical significance of this dwarfing/vigor locus.

An additional 192 individuals were genotyped in fall 2021 to further refine the genetic maps. We thank Dr. Nahla Bassil (USDA-ARS at Corvallis, OR) for DNA extraction and for coordinating the genotyping effort to improve cost efficiencies and quality control. The raw data outputs were received in December 2021, and will be processed, analyzed, and incorporated to improve the current genetic maps.

Objective 4: Expand the pear rootstock parent germplasm

In 2019, the existing rootstock breeding program was supplemented with several diverse *Pyrus* seedlings collected from USDA-ARS, Corvallis to replace a few *Pyrus* parents that died due to fire blight. In subsequent years, no additional rootstock parents were added. Newly propagated precocious selections will be added to the parent set in spring 2022.

Objective 5: Evaluate B × A and B × C selections

The 14 unique selections (‘Bartlett’ × ‘d’Anjou’ and ‘Bartlett’ × ‘Comice’) grown in triplicate (total of ~45) are being maintained at WSU Columbia View orchard. Trees were pruned (except central

leaders) and trained to induce fruit production. We thank Dr. Stefano Musacchi for his advice on training these trees.

Trees are just starting to fruit with six accessions bearing fruit in fall 2021. Fruit count (total: 27) and weight were recorded. Ten of the 14 selections did not bloom in spring 2021. In the next three years, more information on dwarfing and precocity will be collected to determine which rootstocks would be discarded based on low dwarfing potential and non-precocious bearing. In addition, fruit size, texture and skin finish will be evaluated, as relevant.

In winter 2019-2021, the trees were phenotyped for various vigor-related traits. Consistent segregations for vigor-related traits were observed among triplicated selections; however, it is still too early to draw meaningful conclusions. High correlation coefficients were reported between scion trunk cross-sectional area and scion tree height of multiple years. These scion traits were negatively or non-correlated with rootstock seedling traits (collected in 2012), suggesting that compact rootstock seedling stature is not indicative of dwarfing potential.

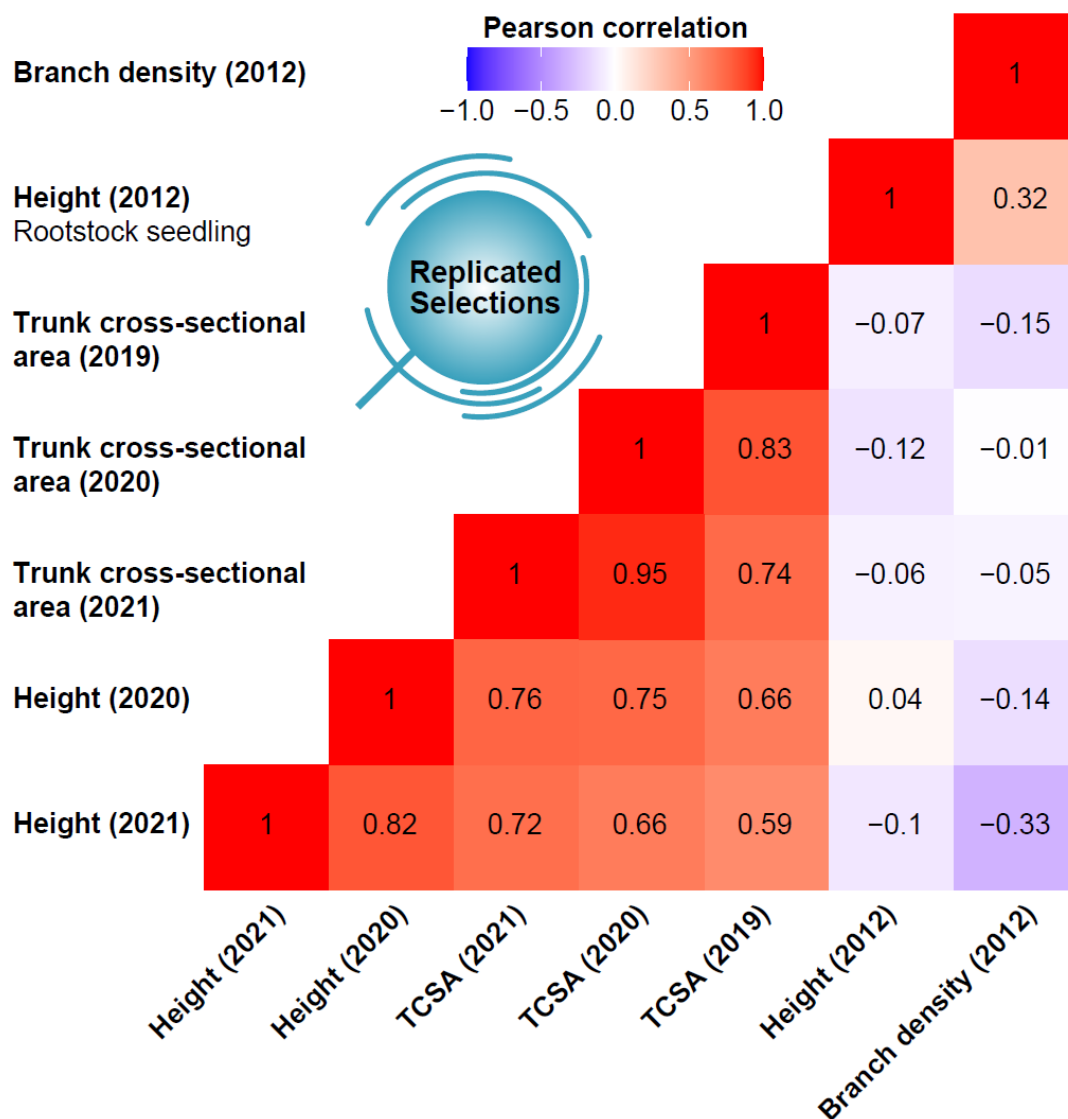


Figure 2: Correlation analysis of replicated selections of ‘Bartlett’ × ‘d’Anjou’ and ‘Bartlett’ × ‘Comice’. Scion traits (height and trunk cross-sectional area) of 2019-2021 are highly correlated, but are negatively or non-correlated with rootstock seedling traits collected in 2012.

OUTREACH

- Soon Li Teh presented “Pear rootstock breeding program” at the WSU Sunrise Research Farm Extension Field Day at Rock Island, WA on August 7, 2019.
- Soon Li Teh presented “Initiating pear rootstock breeding at Washington State University” at the 2019 Annual Meeting for National Association of Plant Breeders (NAPB) at Pine Mountain, GA on August 25 – 29, 2019.
- The WSU pear rootstock breeding program was featured as a Good Fruit Grower article, “Rooting out Solutions for Pear Growers” on September 2019 Issue (<https://www.goodfruit.com/rooting-out-solutions-for-pear-growers/>).
- Soon Li Teh and graduate student, Zara York presented an overview of pear rootstock breeding at the WSU Tree Fruit Breeding 101 – Extension Field event at Orondo, WA on October 24, 2019.
- Zara York presented “Advancing genetic resources for pear rootstock breeding” Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA in December 2019.
- Soon Li Teh presented “Initiating pear rootstock breeding at Washington State University” at the 10th Rosaceae Genomics Conference (virtual/online) on December 9 – 11, 16 – 18, 2020.
- Zara York presented “Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations” at WSU Department of Horticulture – Research Proposal Expo via Zoom on April 21, 2020.
- Zara York, Soon Li Teh and Kate Evans presented “Phenotypic and genetic characterization of dwarfing-related traits in bi-parental pear rootstock populations” at the 2020 Annual Meeting for National Association of Plant Breeders via Zoom on August 18, 2020.
- Soon Li Teh led a pear discussion group during a “U.S. Nationwide Pear Researcher Meeting” (virtual format) coordinated by Dr. Jessica Waite on March 9-10, 2021.
- Soon Li Teh gave a field tour on “Overview of WSU apple scion and pear rootstock breeding programs” to students of the Cascade Christian Academy High School at Orondo, WA on May 4, 2021.
- Kate Evans hosted the WSU cohort of ‘FACT: Research Experience for Undergraduates on Phenomics Big Data Management’ at WSU Columbia View orchard, describing the rationale and process of pear rootstock breeding on July 9, 2021.
- Soon Li Teh delivered a guest lecture on “Pear rootstock breeding” at WSU Department of Horticulture (*HORT 503* – virtual format) on November 15, 2021.
- Soon Li Teh and Tory Schmidt (WTFRC) facilitated a panel discussion on “Evaluating new rootstocks for pears” at NCW Pear Day on January 20, 2022.

EXECUTIVE SUMMARY

Project Title: Pear Rootstock Breeding

Key words: breeding, dwarfing, precocious, *Pyrus*, rootstock

Background: The pear industry lacks dwarfing rootstocks that can transform orchard structures to enable application of new technologies to improve efficiencies. This project aimed to build on previous breeding progress to develop a long-term, dedicated pear rootstock breeding program at the WSU-TFREC, Wenatchee. Evaluation of rootstock populations began in this project, which also included the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing. In summary this project encompassed: (1) developing seedling populations to produce new rootstocks; (2) validating published dwarf and dwarfing markers for potential use in selection; (3) conducting marker-trait association for dwarfing traits; (4) expanding pear rootstock parent germplasm; as well as (5) evaluating B × A and B × C selections.

Outcomes and significant findings: Approximately 2,000 seedlings that were budded with d'Anjou are being maintained at the WSU Columbia View orchard. Evaluation of their dwarfing potential is ongoing, with up to 3 years of rootstock and scion trait data collected. Ten preliminary precocious seedlings with medium to high early dwarfing effect were identified and micropropagated. Breeding parents were tested with published dwarf and dwarfing DNA markers. None of the reported dwarf or dwarfing alleles (i.e., genetic copies) were present in the rootstock parent set. Genetic maps of two seedling populations were constructed. A preliminary dwarfing locus (i.e., genetic determinant) was mapped on chromosome 15 in one seedling populations. 45 replicated selections (Bartlett × d'Anjou and Bartlett × Comice) were phenotyped for vigor-related traits, which were highly correlated. Trees are just starting to fruit with six accessions bearing fruit in fall 2021.

Future directions: Current seedling populations will continue to be evaluated to produce more robust phenotypic data that can be integrated with existing genotypic information to facilitate future selection of desirable rootstocks. Additional years of phenotypic data will be used to: (1) validate the preliminary dwarfing locus on chromosome 15; and (2) identify other dwarfing and/or precocious locus/loci. The ten precocious seedling candidates will be replicated and planted at the WSU-Sunrise orchard for use as future crossing parents. More fruit are expected from the B × A and B × C selections, which will be evaluated for fruit quality and skin finish.