Project Title: Pear Rootstock Breeding PR-22-102

Report Type: Final Project Report

Primary PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-293-8760
Email: kate_evans@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Co-PI 2: Soon Li Teh (moved to new position at UMN 10.2.23)
Organization: WSU TFREC
Telephone: 509-293-8813
Email: soonli.teh@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee WA 98801

Cooperators:
Amit Dhingra (Texas A&M University), Jessica Waite (USDA-ARS Wenatchee, WA), Lauri Reinhold (USDA-ARS Corvallis, OR), Nahla Bassil (USDA-ARS Corvallis, OR), Stefano Musacchi (WSU-TFREC)

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $100,592
Total Project Request for Year 2 Funding: $101,401
Total Project Request for Year 3 Funding: $101,025

Other related/associated funding sources: Awarded
Funding Duration: 2022
Amount: approximately $6,000
Agency Name: USDA ARS
Notes: Summer intern (Edwin Polanco) "FACT: Research Experience for Undergraduates on Phenomics Big Data Management." (PI: Sankaran). Award covered stipend plus travel and housing.

Funding Duration: 2022 - 2025
Amount: various
Agency Name: Pome fruit breeding program royalties
Notes: apple royalties used to supplement for e.g. conference travel costs, publication fees, equipment, collaborative genetics/genomics research with cooperator Waite including graduate student Ramesh Pilli.

Funding Duration: 2023
Amount: $147,827
Agency Name: NNII
Notes: orchard (CV) infrastructure and equipment which will benefit both pear rootstock and apple scion breeding programs

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Kate Evans
Organization Name: WSU-TFREC
Contract Administrator: Waylan Safranski
Telephone: 509 335 2723
Contract administrator email address: wski@wsu.edu

Item	2022	2023	2024
Salaries	$53,144.00	$55,270.00	$57,481.00
Benefits	$17,507.00	$18,207.00	$18,936.00
Wages	$6,955.00	$7,233.00	$7,522.00
Benefits	$4,365.00	$4,539.00	$4,721.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$12,890.00	$9,890.00	$5,890.00
Travel	$3,080.00	$3,080.00	$3,080.00
Plot Fees	$2,651.00	$3,182.00	$3,395.00
Miscellaneous	$0.00		
Total	$100,592.00	$101,401.00	$101,025.00

Footnotes: Salaries for research assistant professor (Teh) and then Research Associate (Cain) as the point person for pear rootstock; Wages for time-slip labor for orchard management and trait phenotyping; In-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. Conduct marker-trait association for rootstock-conferred traits in seedling populations
3. Validate stability/repeatability of preliminary dwarfing locus
4. Maintain a relevant pear rootstock parent germplasm
5. Evaluate B × A and B × C selections

A major bottleneck to high-density pear production in the Pacific Northwest is the lack of dwarfing, precocious, cold-hardy rootstocks. Such rootstocks can transform the industry enabling high-density pear production. A foundational project (PI: Evans; "Pear Rootstock Breeding"; PR-09-905) established a collection of diverse parental germplasm for use as crossing parents. The first pear rootstock seedling populations were produced in PR-15-105 "Pear Rootstock Breeding" (PI: Evans) (see Fig 1 for proposed breeding timeline). Evaluation of these populations began in project PR-19-108, which also included the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing. This project aimed to build on recent (and concurrent) research to further develop a long-term dedicated pear rootstock breeding program at the WSU Tree Fruit Research and Extension Center, Wenatchee, WA. Research effort focused on evaluating current seedling populations for selection and collecting robust phenotypic data that could be integrated with existing genotypic information to facilitate efficient future selection of desirable rootstocks.

SIGNIFICANT FINDINGS

- Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits in 2022-2025.
- Ten precocious seedlings that were previously micropropagated (10 replicates per seedlings) are being maintained in the WSU TFREC hoop house to add to the parent germplasm set.
- Forty-two seedlings from the 2016 seedling families (including some of the previously micropropagated individuals) were selected for propagation for further evaluation and possible Phase 2 inclusion.
- Two dwarfing loci were identified (on chromosomes 5 and 15); analysis was completed to characterize their haploblocks and their relative contributions to vigor reduction.

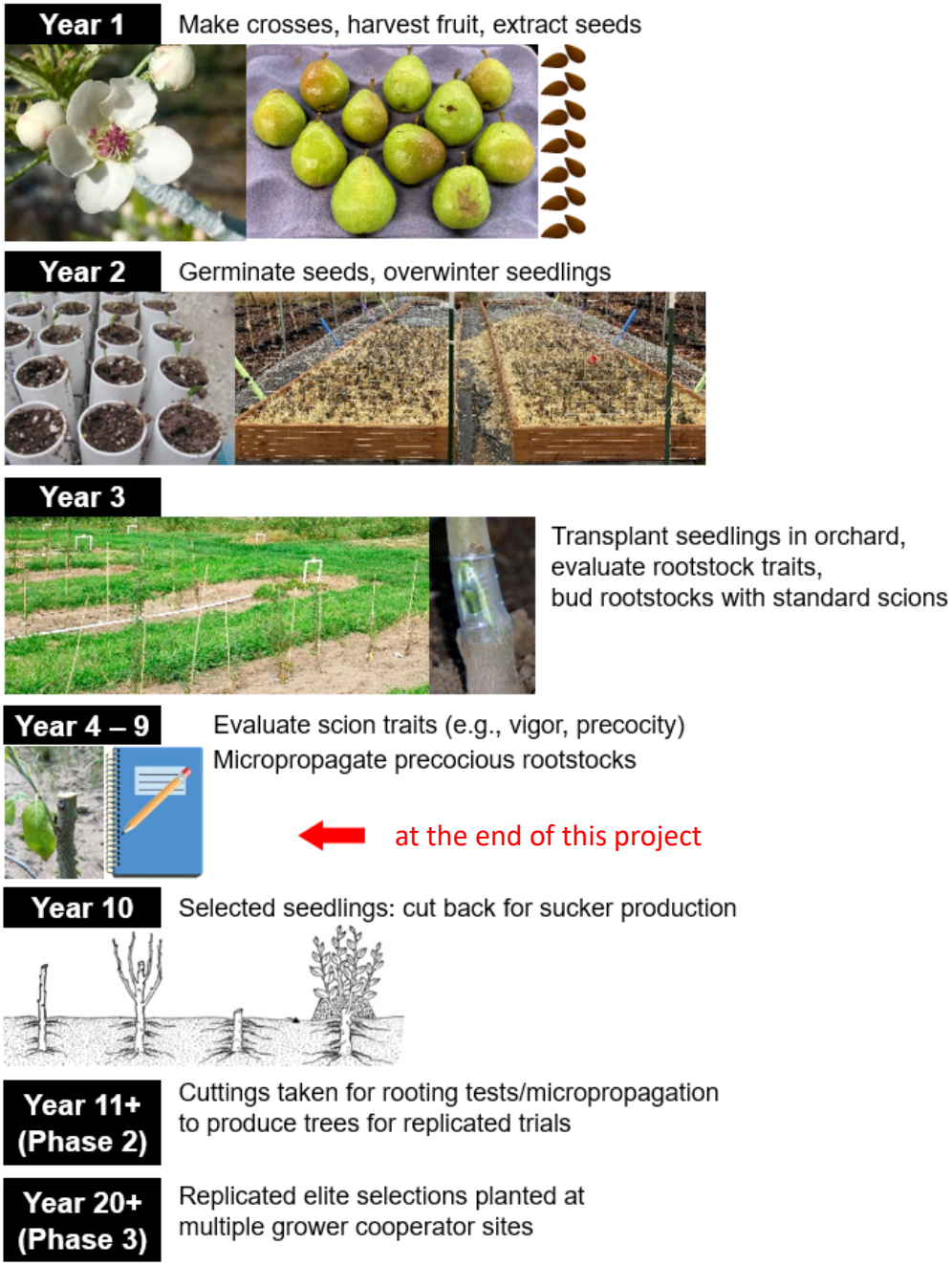

Figure 1: Timeline overview of the WSU pear rootstock breeding program in developing new dwarfing pear rootstocks.

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 (2020-22)	Branch angle (2020-22) Floral bud count (2021-24) Internode length (2020-22) Scion growth (2020-22) Trunk diameter (2020-23) Budbreak (2021-23)
2017	~320	Branch angle (2020) Presence of spine (2020)	Branch angle (2023-24) Floral bud count (2023-24) Internode length (2022) Scion growth (2022-24) Trunk diameter (2022-24) Budbreak (2022-24)
2019	~1,000	Branch angle (2022) Presence of spine (2022)	Branch angle (2024) Floral bud count (2023-24) Internode length (2023) Scion growth (2023-24) Trunk diameter (2023-24) Budbreak (2023-24)

Table 1: Data collection of various rootstock seedling and scion (d'Anjou) traits for breeding and selection.

Bloom data was collected for all populations each spring, however due to risk of fire blight infection, once bloom was recorded, it was removed.
Evaluation of the 2016 seedling families was completed and forty-two individuals were selected in summer 2024, moving ahead of the expected timeline shown in Figure 1. Tissue was sent to Qualterra for micropropagation. In addition, seedling trees were cut back to below the graft union and mounded with sawdust to encourage production of rooted suckers. The aim is for sufficient material to test for fire blight resistance in the greenhouse and to establish a subset of individuals into a Phase 2 replicated trial in a subsequent project.
Approximately 1400 new pear seeds produced in 2023 are currently germinating and will be screened for resistance to fire blight in the greenhouse in spring 2025.

Objective 2: Conduct marker-trait association for rootstock-conferred traits in seedling populations

Two dwarfing loci were identified (on chromosomes 5 and 15) in two of the 2016 seedling families. Further analysis was completed to characterize their haploblocks and their relative contributions to vigor reduction. [A haploblock is a section of DNA that tends to be inherited as a unit rather than frequently be rearranged during meiosis.] Each dwarfing haplotype accounted for 30% to 50% reduction in vigor ($p < 0.05$). Combined haplotype analysis

showed that one dwarfing locus was sufficient to significantly reduce vigor. Presence of two dwarfing haplotypes further reduced vigor by a total of 50% to 70% ($p < 0.05$).

Objective 3: Validate stability/repeatability of preliminary dwarfing locus
We are continuing to collaborate with Dr. Waite (USDA-ARS, Wenatchee) to add precision to the DNA region associated with dwarfing, using a new computational tool (Khufu) to identify genetic variants in our data set. Progress has been slower than expected with Hudson Alpha, however we recently started collaborating with Dr. Gottschalk (USDA-ARS, Kearneysville) and hope to leverage his experience with similar data sets.

Objective 4: Maintain a relevant pear rootstock parent germplasm
The existing rootstock parent germplasm continues to be maintained at WSU Sunrise orchard. No new material has been added although ten precocious seedlings from the 2016 families have been micropropagated with a view to add to the parent collection.

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. Musacchi for his advice on training these trees.
Fruit yield and quality data has been collected for both the 2023 and 2024 season, which together with vigor data should enable final selection in spring 2025.

Executive Summary

Project title: Pear Rootstock Breeding

Key words: dwarfing; precocious; *Pyrus*

Abstract:

The lack of dwarfing, precocious, cold-hardy rootstocks is a major bottleneck to high-density pear production in the Pacific Northwest. Such rootstocks can transform the industry enabling high-density pear production. This project aimed to build on recent (and concurrent) research to further develop a long-term dedicated pear rootstock breeding program at the WSU Tree Fruit Research and Extension Center, Wenatchee, WA. Research effort focused on evaluating current seedling populations for selection and collecting robust phenotypic data that could be integrated with existing genotypic information to facilitate efficient future selection of desirable rootstocks.

Approximately 2,000 *Pyrus* seedlings were evaluated for scion and rootstock vigor traits over the 2022-2025 period. Forty-two seedlings from the oldest seedling families (2016) were selected for propagation for further evaluation, with a view to including some of them in a future replicated Phase 2 trial. Final selections on an earlier small set of potential rootstocks (B × A and B × C trees) will be made in spring 2025; these would also need to be evaluated in a full replicated trial.

Data from the oldest seedling block was used to identify two dwarfing loci (areas of the genome on chromosomes 5 and 15); analysis was completed to characterize their relative contributions to vigor reduction. Further data mining is on-going to add precision to the regions identified as they contain many genes, the first steps toward establishing necessary genotyping resources to inform breeding for dwarfing.