

**FINAL PROJECT REPORT**  
**WTFRC Project Number: PR-19-101**

**YEAR: 3 of 3**

**Project Title:** Evaluating dwarfing capacity of 65 diverse pear germplasm accessions

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**Cooperators:** David Neale, UC Davis; Joseph Postman, USDA-ARS Corvallis pear germplasm repository; Rick Sharpe, WSU Pullman and Soon Li Teh, WSU TFREC; Jessica Waite, USDA Wenatchee

**Budget: Year 1:** \$40,081

**Year 2:** \$40,323

**Year 3:** \$40,116

**Other funding sources: Awarded**

**Amount:** \$73,459 (2017 – 2019)

**Agency Name:** Fresh & Processed Pear Committee Research

**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.

**Other funding sources: Awarded**

**Amount:** \$322,003 (2019 – 2022)

**Agency Name:** Fresh & Processed Pear Committee Research

**Notes:** “Pear Rootstock Breeding” (PI: Evans; Co-PI: Dhingra)

Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

**WTFRC Collaborative Expenses:** None

**Budget**

**Organization Name:** Washington State Univ

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<b>Item</b>	<b>2019</b>	<b>2020</b>	<b>2021</b>
<b>Salaries<sup>1</sup></b>	22,909	23,825	24,778
<b>Benefits</b>	8,172	8,498	8,838
<b>Supplies<sup>2</sup></b>	5,000	4,000	2,500
<b>Travel</b>	1,000	1,000	1,000
<b>Plot Fees<sup>3</sup></b>	3,000	3,000	3,000
<b>Total</b>	40,081	40,323	40,116

1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants

2 – Greenhouse soil and supplies, tissue culture consumables, vessels, chemicals and supplies, grafting supplies

3 – Greenhouse space usage fee per year

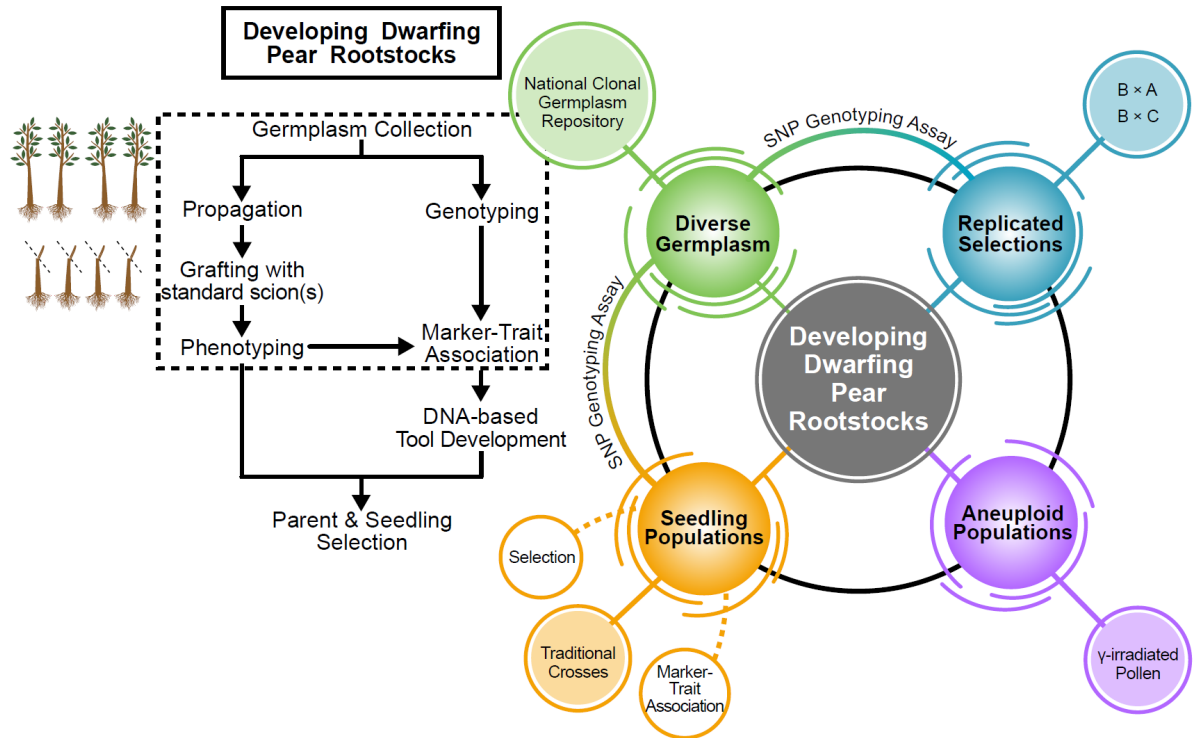
## **RECAP OF THE ORIGINAL OBJECTIVES**

1. Complete the initiation, multiplication and rooting of the remaining germplasm accessions in tissue culture and greenhouse.
2. Graft 5 clones from each of the accession with scion wood from ‘Bartlett’ and ‘Anjou’. Use ‘OH×F 87’ as a control.

This is a synergistic project aimed towards developing dwarfing, precocious and disease resistant pear rootstocks. It involved collection of 65 diverse accessions of pear germplasm selected for the desirable traits. These accessions were collected with support from a previous project (PI: Evans “Pear rootstock breeding”; PR-15-105). The goal of this project was to establish all the remaining accessions in tissue culture as well as establish ten clones from each accession so that they could be used for grafting and subsequent phenotyping for dwarfing.

## **SIGNIFICANT FINDINGS**

- Of the 65 accessions 61 were successfully cloned either in the micropropagation system or in soil. The endophytic contaminants proved fatal for four of them despite multiple attempts during the course of the project.
- Due to the need for repeated establishment and management in tissue culture and greenhouse, the entire focus was centered around objective 1.
- Given the wide range of diversity of the accessions, the growth rates, caliper and architecture of the clones in soil are highly variable at the time of this report.
- The accessions were collected multiple times in 2019 and 2021, therefore the clones in soil derived from different accessions are not of the same age. Samples were not collected in 2020 due to COVID.
- The asynchronous establishment of the accessions in tissue culture over the years and their subsequent establishment in soil has resulted in a population that will need additional time to reach the requisite caliper for grafting.
- For most accessions, there are more than ten clones each in soil and for others back up material is available in tissue culture.
- Both the cultures and the replicated clones will be provided to Dr. Jessica Waite at USDA to continue the maintenance of the genetic material.



**Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks.** This illustration outlines the four synergistic projects. This project focuses on the diverse germplasm accessions. Replicated populations of the diverse germplasm both in soil and tissue culture will be transferred from the Dhingra program to the Waite USDA lab in 2022.

## RESULTS AND DISCUSSION

### **Objective 1. Complete the initiation, multiplication and rooting of the remaining germplasm accessions in tissue culture and greenhouse.**

Of the 65 accessions, 61 were successfully established either in tissue culture or in soil as a source of clean and genetically true-to-type plant material. These diverse accessions were previously genotyped using the Pear SNParray produced as part of a collaborative project with UC Davis - PI: Neale, Co-PI Dhingra, “Development of marker-based breeding technologies”; PR-14-111.

Throughout the project, heavy bacterial and fungal infestation in plant material derived from the germplasm repository necessitated repeated initiation of the accessions in tissue culture. Due to COVID, no new initiations were done in 2020.

The plant material that has gone through the process of initiation is maintained either in tissue culture or in the greenhouse. The tissue culture material is further divided into dormant (39 deg F) or active growing (75 deg F). This allows for management of the material and avoiding any cross contamination. We observed that the endophytic microbes in clean material manifested after 6-8 months and destroyed some of the cultures.

Due to the repeated initiations, the plant material in the greenhouse is also divided into two categories. There is a set of clones that is older than 1 year and another that is younger. These plant materials are growing well but their growth rate, caliper and architecture are highly variable.

The replicated clones and plant material in tissue culture will be transferred to the Waite USDA lab in Wenatchee in 2022 to continue this project.

A summary of status of each accession in terms of number of plants in the micropropagation process, soil or in the cold as of January 20<sup>th</sup> is presented in Table 1.

**Table 1: Status and number of clones available for all the accessions representing a diverse set of *Pyrus spp.*** Four rows highlighted in gray represent the accessions that failed to be established despite multiple attempts.

Sample#	Row USDA Corvallis	position	# of boxes in TC @4C	Total # of shoots in TC @4C	# of boxes in TC @ 24C	Total # of shoots in TC @ 24C	# of rooted saplings 1 year+	# of small rooted plantlets < 1 year old	Total number of rooted clones in soil
1	NF 23	1	2	18	0				0
2	NF 23	15	0	0	0		20		20
3	NF 23	14	0	0	2	10+	9		9
4	NF 24	11	0	0	2	10+	12	11	23
5	NF 25	8	0	0	2	10+	17	5	22
6	NF 28	9	0	0	3	15+	15		15
7	NF 30	4	0	0	0			8	8
8	NF 31	16	1	5	0				0
9	NF 32	13	2	18	0				0
10	NF 33	4	0		0		13		13
11	NF 34	2	0	0	0				0
12	NF 34	7	0	0	0				0
13	NF 52	1	0	0	0	10+	17	9	26
14	1	17	0	0	2	10+	10		10
15	1	21	0	0	2	10+	30		30
16	2	3	2	12	1	6	7	6	13
17	2	23	0	0	3	15+	18	6	24
18	2	27	0	0	1	6+	15	3	18
19	3	15	0	0	0		8	2	10
20	3	25	2	16	0				0
21	4	19	0	0	0				0
22	4	21	0	0	2	10+	10	9	19
23	4	45	1	7	1	5		1	1
25	5	11	0	0	2	8	19	4	23
26	5	21	2	6	0				0
27	6	45	0		0		11		11



**Objective 2. Graft 5 clones from each of the accessions with scion wood from 'Bartlett' and 'Anjou'. Use 'OH×F 87' as a control.**

Due to variable growth patterns and the need to repeatedly initiate plant material, the goals of this objective were not achieved. The OH×F 87 clones did achieve the desired caliper for budding. The non-domesticated germplasm presented the unexpected challenge of much slower growth rate and a highly variable architecture. It is expected that the Waite USDA labs will utilize this material to continue the budding work. The Waite USDA lab also plans to phenotype this population for rooting potential as part of another complimentary project.

## 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.
- Soon Li Teh presented “Initiating pear rootstock breeding at Washington State University” at the 10<sup>th</sup> Rosaceae Genomics Conference (virtual/online) on December 9 – 11, 16 – 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 delivered a guest lecture on “Pear rootstock breeding” at WSU Department of Horticulture (*HORT 503* – virtual format) on November 15, 2021.
- Amit Dhingra visited Fowler Nurseries, Sierra Gold Nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in November 2019.
- Amit Dhingra presented a seminar at Pairwise Inc. in North Carolina regarding pear genomics and rootstock breeding in September 2019.
- Amit Dhingra hosted farmers from Yakima in February 2020 and shared details about the pear rootstock breeding project
- Amit Dhingra visited nurseries and informed them regarding horticultural genomics work including pear rootstock breeding in the PNW in February 2020
- Amit Dhingra shared the approaches of pear rootstock breeding using greenhouse based generation cycling as part of a AG2PI workshop in July 2021
- Amit Dhingra shared the pear rootstock research at the annual Fruit Conference in New Braunfels, TX in October 2021
- Amit Dhingra provided an update on pear rootstock research at the Texas Nursery and Landscape Association Lone Star Horticulture Forum January 2022

## EXECUTIVE SUMMARY

**Project Title:** Evaluating dwarfing capacity of 65 diverse pear germplasm accessions

**Key words:** *Pyrus*, genetic diversity, germplasm, dwarfing, precocious

*Background:* There is a need for dwarfing rootstocks for *Pyrus* to enable high density production to enhance orchard profitability. However, very little is known about how a dwarfing rootstock might control vigor, which makes selecting new improved rootstocks challenging. In order to enhance the chances of obtaining the genetic donors of dwarfing, 65 germplasm accessions were identified. These accessions also harbored other traits such as disease resistance and stress tolerance.

This project focused on completing the establishment of all 65 accessions in vitro as well as obtain a minimum of 10 clones each for grafting ‘Bartlett’ and ‘D’Anjou’ scions.

*Outcomes and significant findings:* Of the 65 accessions, 61 genotypes have been successfully established either in vitro or in the greenhouse. Each accession has a unique growth rate and architecture as observed by different caliper obtained over the same period of growth. The endophytes in the budwood material collected from the repository necessitated iterative plant material collection.

*Future directions:* The clones that are established in soil as well as in vitro material will be provided to the Waite USDA lab. The plan is to plant them and grow these accessions out for grafting in subsequent seasons. Both the Dhingra program and Waite USDA program plan to continue leveraging this useful germplasm for additional genetics and genomics studies. The phenotyping results from grafting experiments will contribute to the larger project of developing a genetic understanding of dwarfing in *Pyrus*.