FINAL PROJECT REPORT

Project Title: Greenhouse screening of 49 dwarf rootstock candidates

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Cooperators: UC Davis project funded by Pear Bureau NW and Cal Pears.

Total Project Request: Year 1: 34,133 **Year 2**: 19,289 **Year 3**: 20,037

Other funding sources
Agency Name: USDA SCRI Preapplication
Amount Pending: $2,800,000 (2020-2024)
Notes: "Phenotypic and Genomic Characterization of Pyrus Germplasm for Development of Dwarfing Rootstocks for Sustainable Pear Production in the USA" (PI Dhingra, Co- PI Evans). Synergistic project to characterize diverse set of Pyrus germplasm via large scale phenotyping and genotyping.

Agency Name: PNW Pear Bureau
Amount awarded: $322,003 (2019 – 2022)
Notes: "Pear Rootstock Breeding" (PI: Evans; Co-PI: Dhingra, Co-PI: Soon Li Teh)
Synergistic project to develop and establish pear rootstock seedlings to develop dwarfing rootstocks that are suited for high-density pear production.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2017)
Notes: Support for Danielle Guzman, Graduate student in the Dhingra lab.

Agency Name: CA Pear Advisory Board/PNW Pear Bureau
Amt. awarded: $200,000 (2014-2016)
Notes: "Development of Marker-Based Breeding Technologies for Pear Improvement" PI Neale. Synergistic project to develop a database of the genetic variation in the *Pyrus* collection.

Total Project Funding

Item	2017	2018	2019
Salaries[1]	21000	10920	11357
Benefits	10133	5269	5480
Supplies[2]	1000	1000	1000
Travel	500	500	500
Plot Fees[3]	1500	1600	1700
Total	34133	19289	20037

Footnotes:
1 – Support for technical help to multiply rootstock selections, graft with scions and manage plants
2 – Greenhouse soil and supplies
3 – Greenhouse space usage fee per year

OBJECTIVES

1. Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically, and physiologically uniform rooted material for subsequent grafting experiments.
2. Graft 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as control.

The project plan to introduce all selections into tissue culture and establish enough clones for each selection in the greenhouse has been completed. These will then be grafted over with budwood from Bartlett and Anjou. OHxF 87 rootstock will be used as a control. The grafted plants will be grown and maintained in the WSU greenhouse to assess if the dwarfing trait is transmitted to the scion. Data on internode length, height, and ratio between the two; crotch angle will be recorded. Seedlings that impart dwarfing to the scions will be evaluated as rootstock candidates in field trials to be performed after the completion of this project.

SIGNIFICANT FINDINGS

- Budwood from each of the seedling has been successfully established in the micropropagation system.

- The seedlings being cycled through rapid growth process in the greenhouse have achieved a height of 24-40 inches depending on the seedling they were derived from. Besides the variability in height, the caliper is highly variable and at present not suitable for budding.

RESULTS AND DISCUSSION

Objective 1: Establish 49 dwarf seedlings in tissue culture as a source of clean, genetically and physiologically uniform material for subsequent grafting experiments.

The 49 dwarf seedlings were obtained from crosses made in 2013. The growth of these seedlings has been fast tracked using horticultural rapid cycling process which includes providing ecodormancy (cold requirement) treatments in a cold room. The plant material was incubated in the cold for 4 months and has been recently moved to the green house, where the plant material is undergoing active growth (Figure 1). We initiated nearly 50 buds per selection. All of the selections have been successfully introduced and established into the micropropagation system.

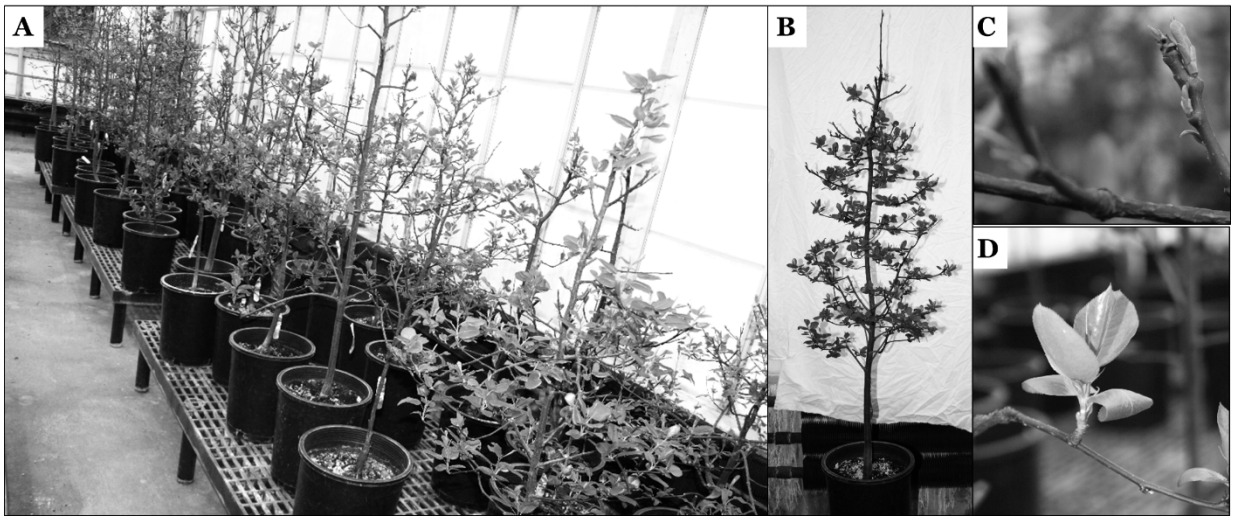

Figure 1: 49 dwarf seedlings in the greenhouse. A. An overview of all the seedlings. B. One of the selections exhibiting a compact growth habit. C and D – actively growing shoots that are being processed to be initiated in the micropropagation system.

Objective 2. Graft or bud 5 clones from each of the seedlings with scion wood from Bartlett and Anjou. Use OHxF87 as a control.

All of the plant material has been cloned and the plants are currently growing in the greenhouse. The plants have reached a height of 24-40 inches depending on the seedling they have been cloned from.

Figure 2: Twenty clones representing each of the 49 seedlings growing actively in the greenhouse.

This project represents one of the four distinct but complementary approaches to establish a foundation for developing dwarfing pear rootstocks (Figure 1). The aim of this project was to evaluate if the dwarf habit of the potentially aneuploid seedlings will transmit to the scion. Promising selections out of the 49 dwarf seedlings could be used as a rootstock selection, or a parent for the pear rootstock breeding program.

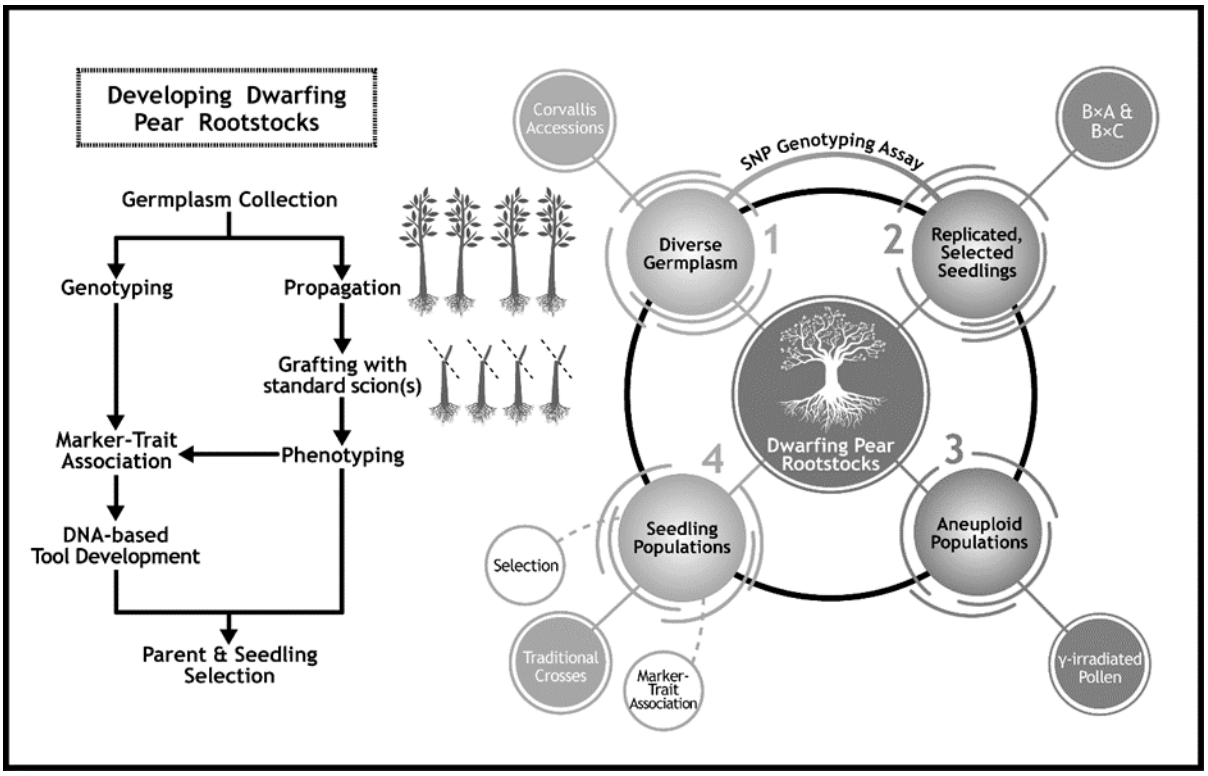

Figure 1: Overview of collaborative efforts involved in developing dwarfing pear rootstocks. This project focuses on germplasm developed using gamma (γ) irradiated pollen and labeled as number 3.

Outreach

- Presentation by Amit Dhingra - 'The foundation for the future of pear production in the PNW' Research News Flash talk at the Washington State Tree Fruit Association Meeting, December 2017.
- 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.
- 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.

- Zara York presented “Advancing genetic resources for pear rootstock breeding” Research News Flash talk at the Washington Horticultural Association Show, Wenatchee, WA December 2019.
- Amit Dhingra presented on pear rootstock research in the Genomic Advances in fruit and vegetable Breeding workshop at the annual Plant and Animal Genome conference at San Diego, CA January 2020.

EXECUTIVE SUMMARY

Project title: Greenhouse screening of 49 dwarf rootstock candidates
Keywords: Dwarfing rootstocks, aneuploidy, micropropagation, genetic diversity, germplasm, seedling population
Abstract: All the seedlings representing 49 dwarfing rootsock candidate were successfully introduced and established into the micropropagation process as a means to preserve the precious genetic material. In addition, a minimum of 20 clones each were established and rooted in the greenhouse. The clones achieved the necessary height, however, the caliper has remained inadequate for budding. The clones will be grown for another season in the greenhouse prior to being budded.

Dwarfing rootstocks have transformed the production and training systems of various tree fruit and nut crops. For instance, in the last 20 years, apple production has increased over 50%, with much of this increase attributed to the adoption of dwarfing rootstocks. The economic impact of dwarfing rootstocks on the U.S. apple industry is estimated to be between $500 million and $1.2 billion per year when comparing low-density and high-density orchards. In the case of pear, dwarfing rootstocks have been used for several decades in Europe, however, in the U.S., 97% of the pear orchards still represent low density plantings with large 3-dimensional pear trees that can reach up to 15 feet in height.

As a foundation for developing dwarfing rootstocks, in 2013, a seedling population was developed from gamma-irradiated pollen to induce aneuploidy in the resulting seedlings. Aneuploidy is a condition where the chromosomal regions of an organism are deleted or duplicated. Utilizing this approach, phenotypes such as dwarfing can be rapidly generated. The resulting seedlings demonstrate a great range of diversity in terms of vigor and branch angle. The next key step is to identify seedlings that induce dwarfing onto the grafted scion

The first aim of this 3-year project was to establish all the aneuploid seedlings in the micropropagation process both to preserve and multiply the material as needed. The second aim was to graft ‘Bartlett’ and ‘D’Anjou’ scions to observe if the compactness of the individual used as a rootstock dwarfed the scion. All of the seedlings have been successfully established in the micropropagation process and continue to be maintained. From this micropropagation repository, 20 clones of each of the seedling have also been established and continue to be grown in the greenhouse. These seedlings are less vigorous and the growth rate is slower than a typical pear seedling. While the clones have reached an average height of 24-40 inches, the caliper needed for successful budding remains to be achieved. The plants will continue to be maintained in the greenhouse and budded in the fall.

The aneuploid population will be used to not only meet the objectives of this project but also to understand which chromosal regions contribute to dwarfing through future project proposals. This material is also being used to support two federal grant proposals - USDA SCRI due March 13th and USDA AFRI – due summer 2020.