FINAL PROJECT REPORT

Project Title: Establishing NW-acclimated Pyrus rootstock breeding material

PI:	Amit Dhingra	**Co-PI**:	Kate Evans
Organization:	Washington State University	**Organization**:	Washington State University
Telephone:	509 335 3625	**Telephone**:	509-663-8181
Email:	adhingra@wsu.edu	**Email**:	kate_evans@wsu.edu

Other funding sources

Agency Name: PNW Pear Bureau
Amt. awarded: $273,253 (2015-2018)
Notes: "Pear Rootstock Breeding" PI Evans, Co- PI Dhingra. Synergistic project to advance the selected pear rootstock seedlings via phenotyping and propagation.

Agency Name: WSU CAHNRS Ignite Program
Amt. awarded: $2500
Notes: Support for an undergraduate student to perform phenotyping and tissue culture of selected seedlings and embryo rescue.

Agency Name: Washington State University Graduate school
Amt. awarded: $34,000 (2016)
Notes: Support for Danielle Guzman, Graduate student – she will perform additional crosses with irradiated pollen in 2016.

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 Request: **Year 1**: 22,000 **Year 2**: 22,185 **Year 3**: 22,992

Budget History:

Item	**2014**	**2015**	**2016**
Wages[a]	13832	14385	14960
Benefits	5577	5800	6032
Supplies[b]	1000	1000	1000
Plot Fees[c]	1000	1000	1000
Total	21,409	22,185	22,992

Footnotes: a. Technical support for plant handling in greenhouse
b. Greenhouse supplies, pots, soil etc.
c. Greenhouse space fees

RECAP OF THE ORIGINAL OBJECTIVES

1. Screen seedlings germinated in 2012 for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions
2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

This project addresses the long-term need for NW acclimated pear rootstocks in the US and is complementary to larger efforts in this direction. In particular, this project focuses on rapid growth of 149 seedlings derived from crosses between 'Bartlett', 'd'Anjou' and 'Comice' and 49 seedlings derived from crosses using gamma irradiate pollen between 'Bartlett', 'd'Anjou', 'Comice' and 'Abate Fetel' in the greenhouse to perform phenotypic screening.

SIGNIFICANT FINDINGS

- Seedlings from crosses made between 'Bartlett', 'd'Anjou' and 'Comice' are in the fifth dormancy cycle. Most seedlings have gone beyond the juvenile phenotype of exhibiting thorns. Two of the "Bartlett" × "d'Anjou" seedlings flowered in spring 2016 in just 4 years from seed germination.
- The technique of rapid cycling through generations to overcome juvenility works in pears and can be utilized for future breeding experiments.
- A selected dwarf subset of the seedling populations have been propagated for small scale replicated trials and will be planted in Wenatchee spring 2017.
- A total of 58 dwarf seedlings have been established from crosses made with gamma irradiated pollen and are ready for evaluation via grafting to see if the dwarfing is transmitted to the scions. A new project has been submitted in 2017 to enable this next step in identifying a dwarf pear rootstock.
- The ratio of number of nodes to height in the irradiated pollen ranges from 0.53 in a 'Bartlett' × 'Abate Fetel' (irradiated) cross to 1.4 in a 'Bartlett' × 'Comice' (irradiated) cross indicating a great degree of spread between vigor and dwarfing.

RESULS AND DISCUSSION

Objective 1: Screen seedlings for growth habit (dwarfing), precocity and floriferousness using rapid growth conditions

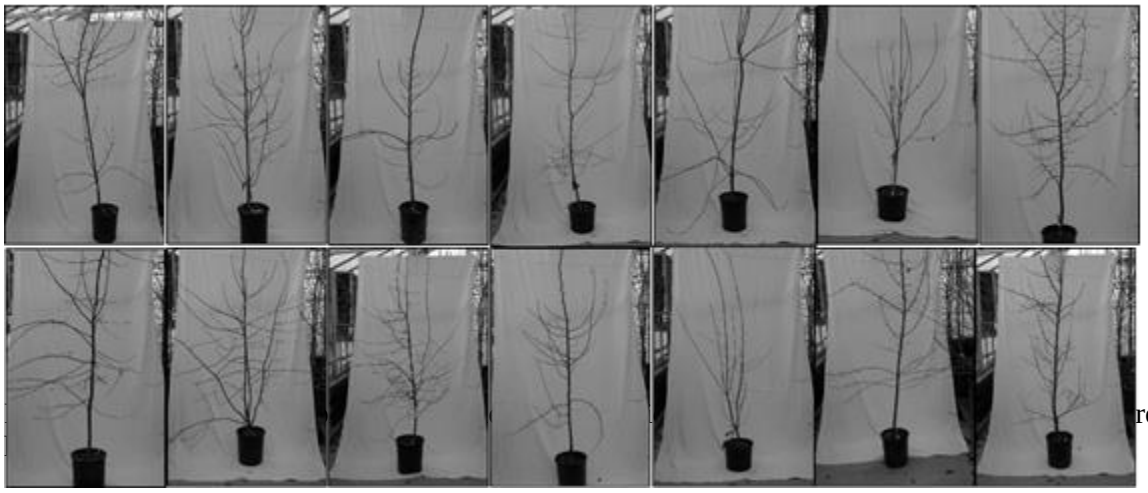
A total of 149 potted trees representing seedlings obtained from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' have been established. The seeds germinated in 2012 are undergoing fifth dormancy cycle and are being maintained in the greenhouse in Pullman. These potted seedlings were scored for node count and height in May 2015. Based on the ratio of number of nodes to height and growth habit, preliminary plant selections were made for desirable seedlings for a complementary project being led by Co-PI Evans. Considerable phenotypic variation was observed in plant habit and wide distribution of ratio of number of nodes to height was recorded. Figure 1 illustrates the extent of variation in habit. A subset of 13 individuals was selected for propagation from crosses 'Bartlett' × 'd'Anjou', and 'Bartlett' × 'Comice' and three trees of each will be planted in a randomized complete block design at the Columbia View orchard, Wenatchee, in spring 2017. The trees will be budded with a standard scion in August 2017. Vigor data will be taken in 2018 and 2019. Currently these plants are in a dormant state (Figure 2) and will be moved to Wenatchee in early spring. A summary of the selected individuals is presented in Table 1.

Table 1 Propagated seedlings for planting in Wenatchee

Cross	Number of plants
B × A 12-13	4
B × A 12-26	4
B × A 12-6	9
B × A 12-21	6
B × A 12-32	3
B × A 12-60	6
B × A 12-9	3
B × C 12-10	4
B × C 12-79	5
B × C 12-69	4
B × C 12-71	2
B × C 12-42	2
B × C 12-37	2

Protocols and approaches developed previously for apples, to accelerate plant growth and cycling through dormancy, continues to be used as a guide for accelerating pear seedling growth in the greenhouse. The plants derived from seeds germinated in 2012 have been taken through five cycles of dormancy since the project was funded. Interestingly, two of the seedlings, B×A 12-44 and B×A 12-19 obtained from "Bartlett" × "d'Anjou" cross produced flowers in 2016 spring (Figure 3). Remaining plants are expected to flower in 2017 spring. This is an exciting outcome as seedlings produced flowers within 4 years of the seeds being germinated.

Seeds obtained from crosses made in the 2013 season were also stratified and were germinated in 12 inch pots filled with potting soil. Once the seedlings were 6 inches tall, they were moved to larger pots. Irrigation and fertilization was performed on a regular schedule standardized for greenhouse plants. For dormancy cycling, these seedlings were moved to the cold room to provide 1000 hours of chilling (ecodormancy) at the first sign of phenotypic markers of shoot growth. Plants were completely defoliated prior to being moved back to ambient growth conditions to initiate vigorous growth. Of the 149 seedlings, only 5 were lost and 144 seedlings continue to be maintained in large pots at the Tukey Orchard, Pullman.



rowth

Figure 3: Flowering observed in two seedlings 12-44 and 12-19 derived from “Bartlett” x “d’Anjou” crosses in 2016.

In summary, this objective has yielded two important and tangible outputs.

1. Identification of 13 seedlings exhibiting the desirable dwarf habit representing plant material that is naturally acclimatized to the PNW region. These seedlings will be planted in Wenatchee for a replicated trial with scions grafted atop these selections. The experiment will be done to evaluate the transmission of dwarfing trait to scions.

2. Identification of two precocious seedlings for which further phenotyping will be performed in the future.

Objective 2. Germinate and subsequent phenotypic screening of seeds derived from irradiated pollen

A total of 49 seedlings derived from 2013 crosses made with irradiated pollen were established. Irradiated pollen was used to generate the foundational trait of dwarfing in pears. This set of plants were cycled through 3 sets of dormancy. The seedlings demonstrate a large degree of variation is size and growth characteristics. The plants were phenotyped for height and number of nodes and the ratio between the two parameters was calculated. It is interesting to note that only two crosses yielded a ratio greater than 1. However there were several seedlings where the ratio was closer to 1. Please refer to Table3. Figure 4 illustrates the diversity in growth habit. These plants are now ready to be grafted and screened for transmission of the dwarfing habit to the scion. A new proposal to evaluate this aspect has been submitted for consideration in 2017.

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Table 3: Ratio of number of nodes/height for seedlings derived from irradiated pollen.

‘Bartlett’ × ‘Bartlett’(irradiated)			
ID	Height(cm)	mber of no	Ratio # nodes/ height
13-6	**71**	**70**	**0.99**
13-4	68	58	0.85
13-1	69	53	0.77
13-2	113	83	0.73
13-5	86	60	0.70
13-7	62	43	0.69
13-3	107	74	0.69

‘Bartlett’ × ‘Comice’ (irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**30**	**42**	**1.40**
13-1	56	42	0.75
13-3	95	71	0.75
13-2	100	67	0.67

‘Comice’ × ‘Comice’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-5	**61**	**60**	**0.98**
13-6	68	61	0.90
13-1	78	60	0.77
13-7	74	52	0.70
13-2	112	77	0.69
13-4	65	43	0.66

‘Abate Fetel’ × ‘Comice’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-1	88	76	0.86
13-2	83	69	0.83
13-4	81	59	0.73
13-1	81	57	0.70
13-3	96	60	0.63

‘Bartlett’ × ‘d’Anjou’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-4	**41**	**45**	**1.10**
13-1	87	80	0.92
13-8	81	73	0.90
13-9	111	97	0.87
13-6	71	62	0.87
13-7	66	57	0.86
13-3	99	68	0.69
13-2	153	103	0.67
13-5	77	51	0.66

‘Bartlett’ × ‘Abate Fetel’(irradiated)			
ID	Height (cm)	Number of nodes	Ratio # nodes/ height
13-2	92	79	0.86
13-3	77	66	0.86
13-9	62	53	0.85
13-14	84	70	0.83
13-8	61	50	0.82
13-11	76	61	0.80
13-5	96	75	0.78
13-13	80	62	0.78
13-6	90	68	0.76
13-15	141	105	0.74
13-10	129	89	0.69
13-4	82	56	0.68
13-1	97	65	0.67
13-16	87	58	0.67
13-7	88	58	0.66
13-17	127	70	0.55
13-12	116	61	0.53

‘Comice’ × ‘d’Anjou’(irradiated)			
ID	Height(cm)	Number of nodes	Ratio # nodes/ height
13-1	55	37	0.67

In summary, this objective has yielded two important and tangible outputs.

1. Generation of several dwarf seedlings derived from material that is naturally acclimatized to the PNW region.

2. Potential rootstocks or rootstock parental material that can be utilized in subsequent rootstock or variety development.

OUTREACH

- Good Fruit Grower article focused on the pear rootstock breeding program was published in September 2015.
- Amit Dhingra hosted the Washington AgForestry leadership group at WSU Pullman; pear rootstock breeding was discussed during a visit to the greenhouses to look at the germplasm.
- Amit Dhingra hosted Doug Hemly (CA pear grower); advances in pear rootstocks was the primary discussion point.
- Kate Evans presented the outline of the breeding program at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk entitled 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.
- Amit Dhingra presented efforts on developing material for pear rootstocks at the Washington State Tree Fruit Association meeting in Yakima December, 2015 in a talk titled, "Smart Plants".
- Kate Evans presented the outline of the breeding program at the Washington State Horticultural Association Show, Wenatchee, December 2016, in a talk entitled 'Update on pear rootstock breeding'.
- Amit Dhingra's article 'The pear industry has unlimited potential and is ripe for a revolution' was published in the Good Fruit Grower, September 2016 (http://www.goodfruit.com/the-age-of-the-pear/ September 14, 2016) and his research on pears was featured in an article in The Atlantic, June 2016 (The push to make pears the new apples). The Atlantic. http://www.theatlantic.com/science/archive/2016/06/battle-of-the-pomes/488687/ June 27, 2016.
- Amit Dhingra presented the role of rootstocks in managing fruit quality in a talk entitled, "Improving Fruit Quality in Pears" at the GS Long Annual Growers Meeting in Chelan, WA on December 15, 2016.

EXECUTIVE SUMMARY

Background: Dwarfing rootstocks are the key to transforming the pear industry. There are 3 primary strategies being pursued to identify a set of dwarf pear rootstocks. 1. Import dwarf rootstocks developed outside of the USA. 2. Make new crosses with imported and domestic parental material and 3. Utilize regionally acclimatized material for traditional and mutation breeding. This project represents the number 3 approach which was initiated by Dhingra and Evans programs prior to obtaining funding for this project in recognition of the urgent need of the USA pear industry.

Outcomes and significant findings: The project resulted in the establishment of 144 F1 seedling population derived from regionally acclimatized parental material. In 2016, 13 seedlings from this group where identified that exhibited the desirable dwarf habit and these seedlings will be planted in Wenatchee for a replicated trial with scions grafted atop these selections. The experiment will be done to evaluate the transmission of dwarfing trait to scions. Two of the 144 seedlings produced flowers and these individuals will be phenotyped in the future. As an outcome of the mutation breeding, several dwarf seedlings were generated which can directly serve as potential rootstocks or parental material that can be utilized in subsequent rootstock or variety development.

Future directions: The seedlings with dwarf habit derived from crosses made with both traditional and irradiated pollen need to be evaluated if they will transmit the dwarf habit to the scion. While a replicated trial has been planned in a complementary project with 13 seedlings from the traditional crosses, additional trials will need to be conducted at multiple locations. One of the exciting outcomes is the establishment of a range of dwarfing habit in seedlings derived from irradiated pollen. It is imperative to evaluate if these seedlings will transmit the dwarf habit to the scions. An economic and rapid way to evaluate that will be to perform the grafting and screening in the greenhouse to select a few desirable candidates.