FINAL REPORT

Project Title: Developing rooting strategies for clonal pear rootstocks

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Other funding sources

Agency Name: California Pear Advisory Board
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Total Project Funding:

Budget History:

Item	**Year 1:**
Salaries	22000
Benefits	12000
Wages	2000
Benefits	
Equipment	
Supplies	2000
Travel	
Plot Fees	
Miscellaneous	
Total	38000

JUSTIFICATION:

The development and use of clonal pear rootstocks was long restricted by the lack of effective and rapid propagation systems. Clonal rootstocks were difficult to propagate both traditionally and *in vitro*. Many promising rootstocks were abandoned because of difficulty with traditional propagation or poor growth *in vitro* (Proebsting, WTFRC reports 2003-7). Development of new pear media makes it possible to produce all types of pears *in vitro* (Reed et al., 2012; Reed et al., 2013). A wide range of both scion and rootstock selections now have excellent growth and multiplication with several mineral nutrient improvements. The types and combinations of mineral nutrients in growth media greatly affected the growth and development of the 20 cultivars tested, and new formulations provide good growth for this diverse group (Fig. 1). Quince selections also propagate well on these media.

Development of effective rooting protocols will allow for *in vitro* or direct rooting of shoots so plantlets can be acclimated, grown up in nurseries and made available to growers. Earlier studies of pear rooting showed that there is great variation in root production *in vitro*; some treatments were highly effective (60-80%), while some pears had low rooting rates or did not root on any of the treatments (Reed, 1995; Yeo and Reed, 1995). These studies were done with pears grown on Murashige and Skoog medium (MS) (Murashige and Skoog, 1962); rooting as well as growth may be improved on medium with a better nutrient formulation. Rooting of propagules is a limiting factor in commercial production of many new rootstock selections.

An improved multiplication medium for propagating clonal rootstocks, PRS medium, is now available, but the final step is to determine effective rooting protocols. We have done some preliminary experiments with 'Horner 51', 'OH×F87' and 'OH×F333' that indicate some genotype related effects of the propagation and rooting media as well as the hormone used for rooting. Once these protocols are developed, the techniques can be used by commercial micropropagation companies to produce a steady supply for the industry. Testing these rooting techniques in a commercial setting will verify the efficacy for transfer to the micropropagation industry. Transferring these outcomes to commercial tissue culture labs, will improve production efficiency and shorten the production period for new pear rootstocks. The best rooting protocols developed in this study could be used to screen additional selections without additional large-scale testing. If the callus induction test proves to be predictive of rooting ability, it could be used to prescreen rooting hormone for each selection.

This is the final portion of research needed to make pear micropropagation viable for rapidly producing clonal rootstock selections for nurseries and growers. All of these techniques will be freely available (no patent or licensing required). These objectives strongly support the pear industry priority of improved propagation techniques for clonal rootstocks.

OBJECTIVES:

1) Determine effect of rooting hormone types and concentrations on callus formation.
2) Compare PRS and MS medium formulations for efficiency of root production.
3) Test rooting protocols on rootstock selections for *in vitro* rooting.
4) Test direct rooting to soilless medium in a commercial setting.
5) Transfer this information to the micropropagation industry for use.

Results

1. Rooting test with 5 pear genotypes with NAA and IBA in DMSO (dimethyl sulfoxide). Five genotypes (9 shoots/ box), 'OH×F69', 'OH×F87', 'OH×F513', 'Horner 51' and 'Pyro 2-33' planted on PRS medium with no growth regulators. **Treatments:** shoots were dipped for 5 seconds in one of the plant growth regulator (PGR) solutions dissolved in DMSO at (0, 1, 5, 10 and 15 mM): **IBA** (indole-3-butyric acid: MW 203.24 g mol^{-1}) and **NAA** (1-naphthaleneacetic acid: MW 186.21 g mol^{-1}).
Results: Controls (no PGR) did not root, NAA was more effective than IBA (Table 1).

Table 1. Percent rooting of five pear rootstocks after dipping in 1-15 mM NAA or IBA dissolved in DMSO. Controls without treatment did not root. Data taken at 4 weeks.

Genotype	NAA (% rooted)				IBA (% rooted)			
	1 mM	5 mM	10 mM	15 mM	1 mM	5 mM	10 mM	15 mM
Horner 51	11.1	66.7	55.6	44.4	33.3	22.2	33.3	44.4
OH×F 69	33.3	66.7	55.6	77.8	11.1	66.7	77.8	33.3
OH×F 87	0.0	50.0	100.0	88.9	0.0	44.4	55.6	88.9
OH×F 513	44.4	75.0	100.0	88.9	11.1	55.6	55.6	66.7
Pyro 2-33	0.0	100.0	88.9	100.0	33.3	66.7	44.4	66.7

1. The second test with higher concentrations of PGR was run with the same genotypes as above and replicated. Five genotypes (9 shoots/ box) × 2 replications × 10 treatments. PRS media with no BA: 80 boxes. IBA and NAA at 0, 5, 10, 15 and 20 mM were tested.

Results: Controls (no PGR) did not root. NAA was more effective than IBA (Table 2).

Table 2. Percent rooting of five pear rootstocks after dipping in 5-20 mM NAA or IBA dissolved in DMSO. Controls without treatment did not root. Data taken at 4 weeks.

Genotype	**NAA** (% rooted)				**IBA** (% rooted)			
	5 mM	10 mM	15 mM	20 mM	5 mM	10 mM	15 mM	20 mM
Horner 51	55	33	72	72	72	77	83	83
OH×F 69	100	100	100	83	83	77	66	94
OH×F 87	94	88	83	100	83	77	72	77
OH×F 513	100	100	94	100	77	100	72	72
Pyro 2-33	88	100	72	77	66	28	61	67

3. Test with polyethylene glycol 400 (PEG 400). Five genotypes planted on PRS medium with no growth regulators with three PGR solutions (NAA 10 mM, IBA 20 mM, and a combination of 5 mM NAA and 10 mM IBA) in 40% PEG 400 in deionized water. Treatments: Dipping for 2 seconds in one of 3 PGR concentrations.

Results: Controls (no PGR) did not root. Shoot quality was better than those rooted with DMSO and most maintained green leaves (Table 3, Figure 1).

Table 3. Percent *in vitro* rooting of five pear rootstocks after dipping in NAA, IBA, or the two combined and dissolved in PEG 400. Controls did not root. Data was taken at 3 weeks.

Genotype	NAA 10 mM	IBA 20 mM	NAA+IBA
Fox 11	100	100	100
Horner 10	78	89	100
OH×F 69	100	100	100
OH×F 87	55	88	73
OH×F 97	100	100	100

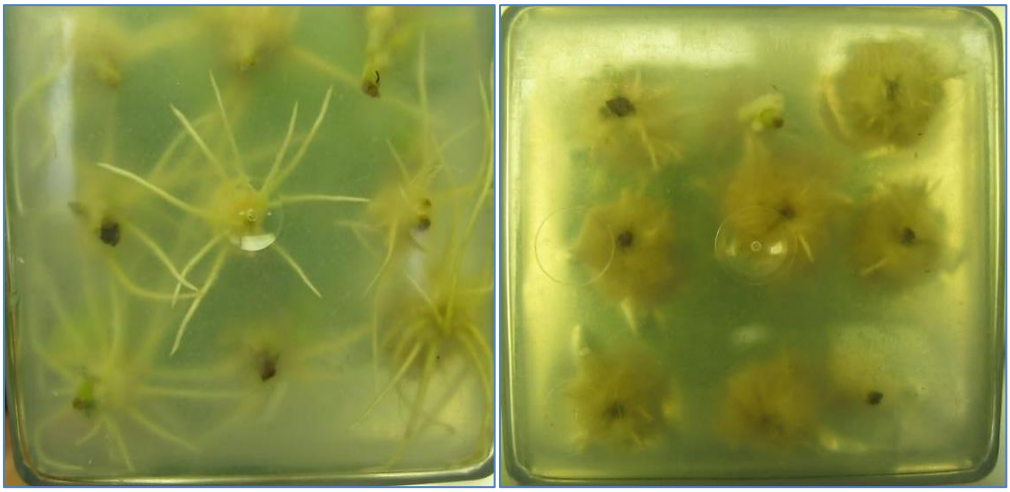

Fig. 1. *In vitro* rooted 'OH×F97' shoots at 3 weeks after treatment with PEG and PGRs. Left: NAA 10 mM, Right: 5 mM NAA + 5 mM IBA.

4. *Ex vitro* direct rooting. Four genotypes ('Horner 4', 'OH×F69', 'OH×F87', and 'OH×F97') were used for *ex vitro* rooting with four replications per treatment. Shoots were cultured on two growth media (MS and PRS) for 4 weeks and transferred to fresh medium for two more times for stabilization (total 12 weeks subculture period). Those shoots grown on the two propagation media were dipped in two rooting hormones at the PGR levels determined in the previous tests (NAA 15 mM and IBA 5 mM combined with NAA 5 mM) dissolved in 40% PEG 400 with DI water, and directly planted in a soilless mix (NA Plants proprietary mix). Shoots were placed under mist in a greenhouse at NA Plants Inc. Rooting was evaluated 4 weeks after planting. Most of the 'Horner 4' treatment could not be evaluated.

Control shoots grown on MS produced an occasional root in some of the genotypes. Control shoots grown on PRS of 'OH×F87' and 'OH×F97' produced some roots and the plants were healthier than those on MS.

Shoots treated with NAA or a mix of NAA and IBA: rooting was variable by treatment and genotype (Fig. 2). 'OH×F97' rooted on all treatments, but the PRS with NAA and IBA had the most roots and plantlets with the best appearance. 'OH×F69' rooted best with the PRS NAA+IBA mix. 'OH×F87' had good rooting on the NAA+IBA mix for both growth media but the PRS grown plants had a better appearance and longer roots. 'Horner 4' rooted very well on the PRS NAA+ IBA mix (the treatments could not be evaluated) (Fig. 3).

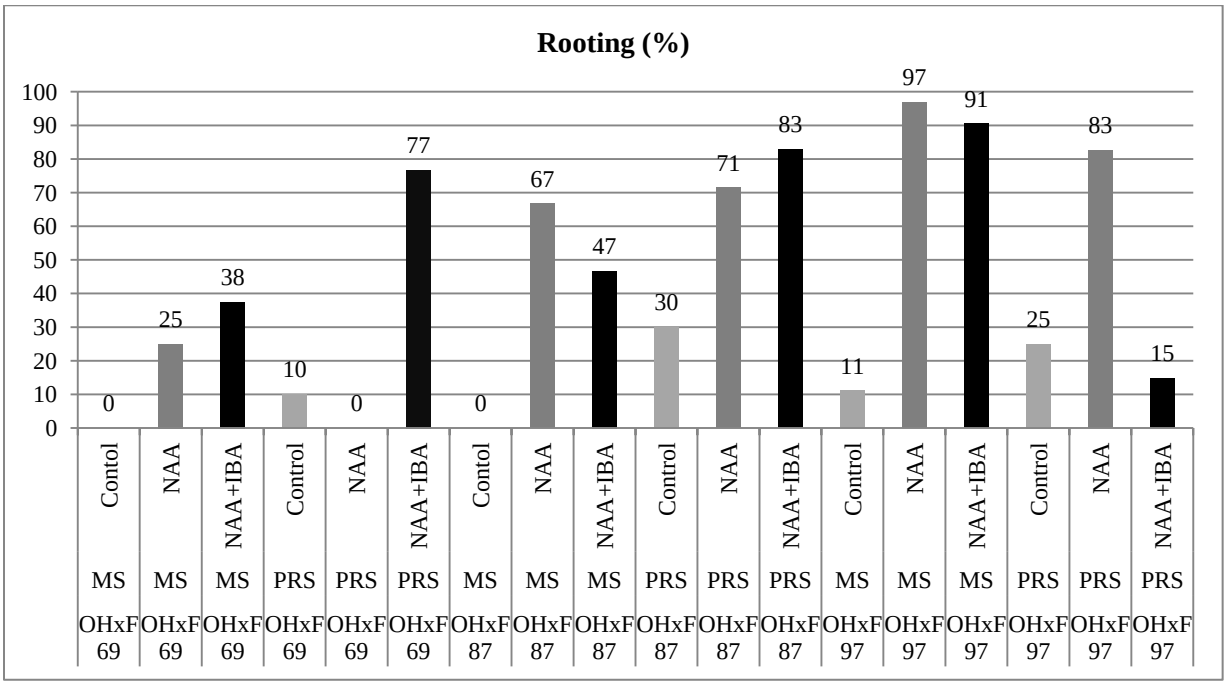

Fig. 2. Rooting of shoots grown on either MS or PRS medium and rooted *ex vitro* with two PGR treatments

- *Blue tag: MS medium with NAA 15 mM*
- *Yellow tag: MS medium with NAA 5 mM plus IBA 5 mM*
- *Green tag: PRS medium with NAA 15 mM*
- *Orange tag: PRS medium with NAA 5 mM plus IBA 5 mM*

Fig. 3. Pictures of *ex vitro* rooted plantlets from the NAA and NAA+IBA treatments on shoots grown on either MS or PRS medium before rooting.

Conclusions:

1. Callus was not always indicative of rooting success
2. *In vitro* rooting was best with NAA for most genotypes when DMSO was used as a solvent.
3. PEG 400 was a better solvent for rooting than DMSO; producing better rooting and healthier plantlets. NAA and IBA combined provided a high rate of rooting for most genotypes.
4. Shoots grown and rooted on the new PRS medium with combined 5 mM IBA and 5 mM NAA produced healthy plantlets with good root systems and a high rooting percentage.

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Executive summary:

The development and use of clonal pear rootstocks was long restricted by the lack of effective and rapid propagation systems. Clonal rootstocks were difficult to propagate both traditionally and *in vitro*. Many promising rootstocks were abandoned because of difficulty with traditional or *in vitro* propagation. Development of new pear media now makes it possible to produce all types of pears *in vitro*. In addition an improved multiplication medium for propagating clonal rootstocks, Improved Pear Rootstock medium (PRS), is now available. A wide range of both scion and rootstock selections now have excellent growth and multiplication with several mineral nutrient improvements through our series of studies and these new media. The types and combinations of mineral nutrients in the growth media greatly affected the growth and development of the 20 pear cultivars tested, and these new formulations now provide good growth for this diverse group.

Rooting is a limiting factor in commercial production of many new root stock selections. Development of effective rooting protocols will allow for *in vitro* or direct *ex vitro* rooting of shoots so plantlets can be acclimated, grown up in nurseries and made available to growers. *In vitro* as well as *ex vitro* testing of efficient rooting techniques in a commercial setting can verify the efficacy for micropropagation industry. The current study determined effective rooting protocols for the rootstock cultivars. Promising rootstocks were grown on the new PRS medium and compared for rooting with rootstocks grown on the standard MS medium. We determined that NAA or NAA combined with IBA were the best plant growth regulators to use for pear rootstocks. We compared root production of shoots propagated on either PRS or MS medium, revealing that PRS medium produced superior plantlets and better rooting for the genotypes tested compared to MS medium. We determined that polyethylene glycol was an efficient solvent for applying the rooting chemicals. In direct rooting tests a combination of NAA and IBA produced excellent rooting on plantlets grown on PRS medium and rooting in a soilless substrate in a commercial setting. Transferring these outcomes to commercial tissue culture labs, will improve production efficiency and shorten the production period for new pear rootstocks. The best rooting protocols developed in this study could be used to screen additional selections without additional large-scale testing. As a whole, these studies provide information that makes pear micropropagation a good option for rapidly producing clonal rootstock selections for nurseries and growers. All of these techniques are freely available through series of publications.

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