FINAL REPORT 2013 **PROPOSED DURATION**: 1 year

Project Title: Developing Rooting Strategies for Clonal Pear Rootstocks

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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. In vitro rooting: First test with 5 pear genotypes with NAA and IBA in DMSO. Five genotypes (9 shoots/ box), OHxF 69, OHxF 87, OHxF 513, Horner 51 and Pyro 2-33. Total 450 shoots. Planted on PRS medium with no growth regulators. **Treatments:** shoots were dipped for 5 seconds in one of the 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 treatment) 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				IBA			
	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
OHxF 69	33.3	66.7	55.6	77.8	11.1	66.7	77.8	33.3
OHxF 87	0.0	50.0	100.0	88.9	0.0	44.4	55.6	88.9
OHxF 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

2. In vitro rooting: Second test with higher ranges of PGR concentration with twice as many shoots (same genotypes) per treatment. Total 900 shoots. 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**				**IBA**			
	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
OHxF 69	100	100	100	83	83	77	66	94
OHxF 87	94	88	83	100	83	77	72	77
OHxF 513	100	100	94	100	77	100	72	72
Pyro 2-33	88	100	72	77	66	28	61	67

3. In vitro rooting: Test with Polyethylene glycol 400 (PEG 400). Five genotypes planted on PRS media 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).

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
OHxF 69	100	100	100
OHxF 87	55	88	73
OHxF 97	100	100	100

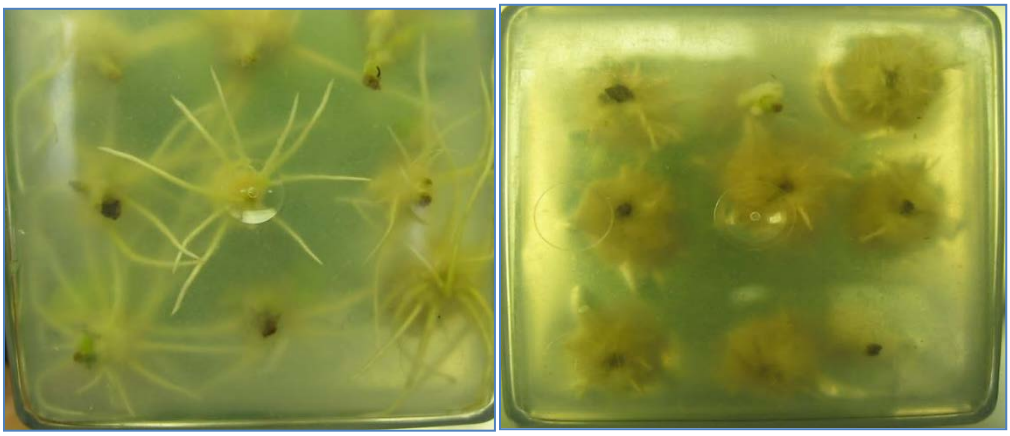

Figure 1. *In vitro* rooted OHxF 97 shoots at 3 weeks after treatment with PEG and PGRs. Left: NAA 10 mM, Right: 5 mM NAA + 10 mM IBA.

4. Direct rooting. Four genotypes (Horner 4, OHxF 69, OHxF 87, and OHxF 97) were used for ex vitro rooting. 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 including callus induction 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 rooting mix (NA Plants proprietary mix). Shoots were placed under mist in a greenhouse at NA Plants Inc. Rooting was evaluated at 4 weeks after planting.

MS controls produced an occasional root in some of the genotypes. PRS controls of OHxF 87 and OHxF 97 produced some roots and the plants were healthier than those on MS (Fig. 2).

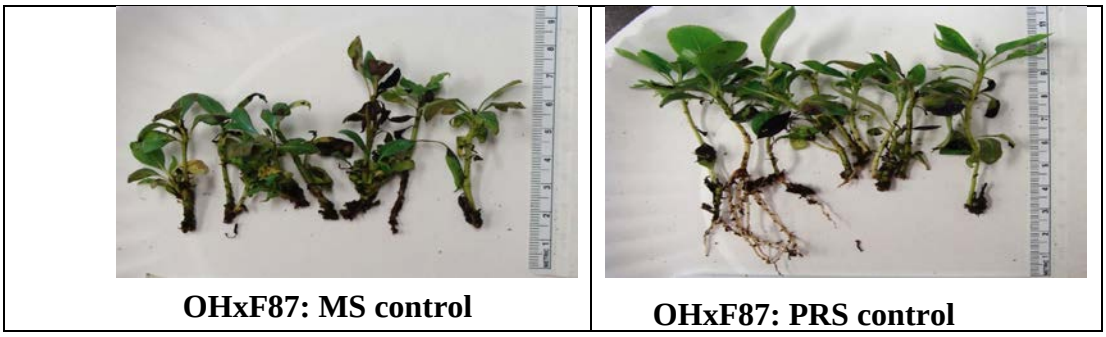

Fig. 2. Control shoots of OHxF 87 stuck for rooting in a soilless mix after growth on either MS medium (left) or PRS medium (right).

Shoots treated with NAA or a mix of NAA and IBA: rooting was variable by treatment and genotype. OHxF 97 rooted on all treatments, but the shoots on PRS with NAA and IBA had the most roots and plantlets with the best appearance. OHxF 69 rooted best the PRS NAA+IBA mix. OHxF 87 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. Control shoots rooted in a few cases.

The growth medium and the PGR used significantly affected rooting (Fig. 3). Plant appearance and rooting was best for plants grown on PRS medium and rooted with the NAA+IBA mix. Response to the other treatments varied by genotype.

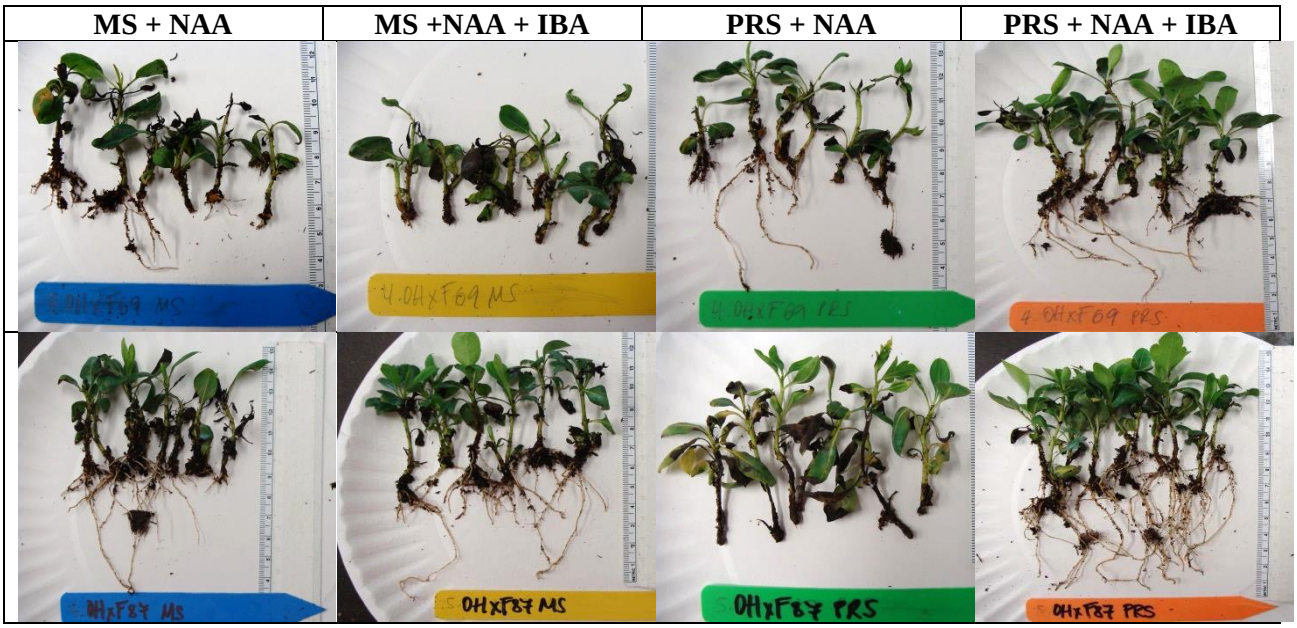

Figure 3. Ex vitro rooting of pear rootstocks after treatment with NAA or NAA+IBA and 4 weeks growth in soilless rooting mix. Some treatments of 'Horner 4' were not evaluated due to accidental loss.

Figure 4. Rooted shoots in soilless mix before removing the mix for measurements.

Conclusions:

1. In vitro rooting was best with 5-15 mM NAA for most genotypes when DMSO was used as a solvent.
2. PEG 400 was a better solvent for rooting than DMSO; producing better rooting and healthier plantlets.
3. PRS medium with 5 mM IBA and 5 mM NAA produced healthy plantlets with good root systems and a high rooting percentage.

Overall the shoots grew better in the new PRS medium and rooted as well as, or better than those on MS medium. We recommend growing all of the rootstock selections on PRS and rooting them with a mix of NAA and IBA.

References

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