

Developing Rooting Strategies for Clonal Pear Rootstocks



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Proposal

1. Compare new medium (PRS) formulations with standard Murashige and Skoog (MS) for efficiency of root production.
2. Test rooting protocols on selected rootstock selections for both *in vitro* and direct rooting.
3. Transfer this information to the micropropagation industry for use.

Outline

1. Project background
2. Medium development for pear rootstocks.
3. Background on pear rooting.
4. Objectives of this proposal.

Pear Micropropagation

- Standard Media are NOT optimal for pear
- Most modifications are “hit or miss” or just test growth regulators.
- Large variation in growth response among cultivars.
- Pears can exhibit a variety of poor growth habits.
 - Callus production
 - Slow or no elongation
 - Slow multiplication or no growth
 - Physiological disorders (shoot tip necrosis, flattened stems)
 - Leaf lesions and edge burn

Examples of poor growth



Optimization Experiments

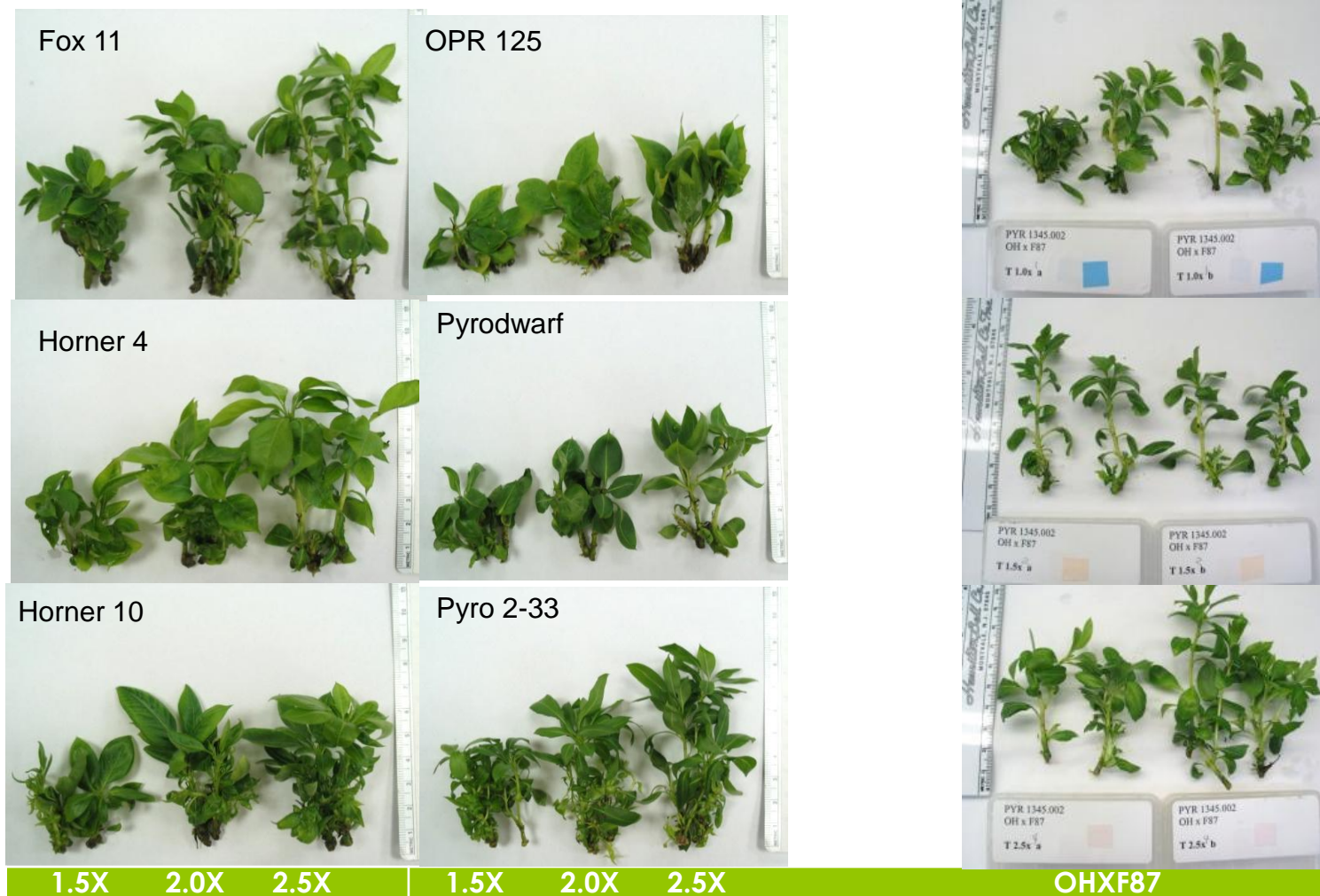
Using MS salts as the base level

1. **Nitrate salts** 0.5 – 1.5X
2. **Ammonium salts** 0.5 – 1.5X
3. **Meso elements** 0.5 – 1.5X
 - $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
 - KH_2PO_4
 - MgSO_4
4. **Minor elements** 0.5 – 4X
 - $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$,
 - $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, KI , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$,
 - H_3BO_3 , $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
5. **Iron** 0.5 – 4X

Medium Development Status

- Determined the main driving factors for improved growth (Mesos, Nitrogen).
- Optimized each of the driving factors
- Developed optimized media for cultivars and species pears.
- Determined that the species and rootstocks respond differently from the scion cultivars.

“Difficult to propagate” rootstocks are easier with increased Mesos (2.5 X MS)



Pear Micropropagation

New medium formulations with changes in mesos and nitrogen produce much better growth than MS for pears in all of these species.

- *P. betulifolia*
- *P. communis*
- *P. calleryana*
- *P. nivalis*
- *P. ussuriensis*



Examples of growth on MS medium



Examples of growth on new media



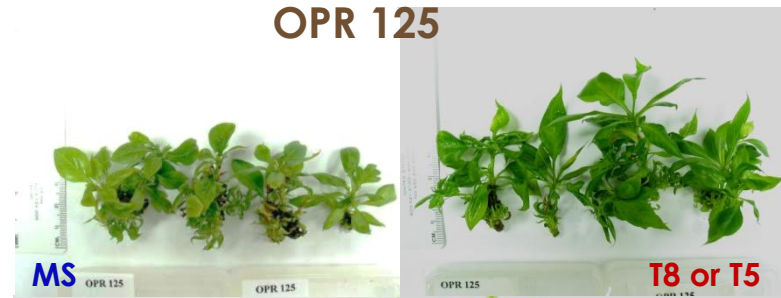
Examples of growth on MS vs new media



G28.120



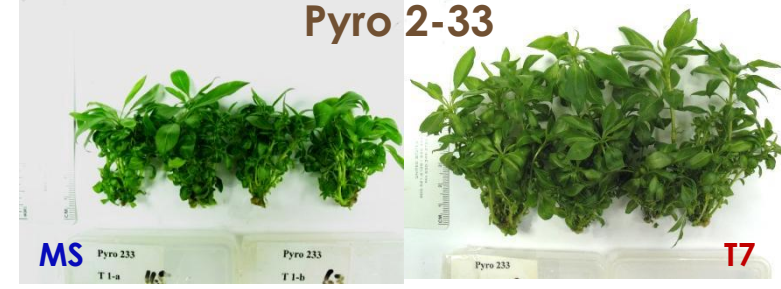
Horner 10



OPR 125



OPR 260



Pyro 2-33

Background on Pear Rooting

1. Need for rooting studies
2. Typical protocols
3. Earlier rooting studies
4. Preliminary studies on new medium

Need for Rooting Studies

1. Large genotype variation in pear response to tissue culture techniques.
2. Multiplication medium can influence root production.
3. Many rootstock selections can't be used because of rooting difficulties.
4. Improved rooting will improve commercial production and availability.

Pear rooting protocols

Response to rooting hormone treatments is very genotype dependent for pears

Auxin treatments: IBA, NAA, IAA

1. Grow one week on treatment, then transfer to hormone-free medium.
2. Dip in hormone and plant in hormone-free medium.
3. Dip in hormone and plant in soil-free mix.

Pear rooting studies

Auxin treatment followed by PGR-free medium (Reed 1995)

Screen of 52 pear accessions with 7 rooting treatments (cultivars, species, 6 rootstocks)

36% rooted >50% with 10 mM IBA dip

40% rooted >50% with 10 mM NAA dip

24% rooted >50% with 10 μ M IBA for 1 week

Pear rooting studies- Rootstocks

IBA or NAA dip treatment or IBA* for one week followed by PGR-free medium (Reed 1995, Yeo and Reed 1995)

- *P. communis* (F.12-173) 61% IBA 56% NAA
- *P. communis* (G.28-119) 61% IBA* 100% NAA
- OH x F 230 >80% with IBA or NAA
- OPR-1 56% IBA*
- OPR 157 24% NAA
- OPR 260 43% IBA*

Preliminary studies

IBA dip rooting trial followed by PGR-free medium (Wada and Reed 2011 unpublished)

- Horner 51 MS-MS 6/6 shoots; others 3/6
- OH x F 87 MS-MS, MS-PRM, PRS-MS 6/6; PRS-PRS 3/6
- OH x F 333 PRS-PRS 86/100 shoots

All rooted shoots survived and grew in the mist bed.



Objectives

1. Determine effect of rooting hormone 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 soil-free medium in a commercial setting.
5. Transfer this information to the micropropagation industry for use.

Objective 1

Determine effect of rooting hormone concentrations on callus formation

- Moderate callus formation (1-5 mm) is ideal for root induction
- Response with IBA, NAA.
- Determine concentration needed for each genotype and each hormone.

Objective 2

Compare PRS and MS medium formulations for efficiency of root production

- Grow rootstock selections on MS and PRS
- Compare growth medium and rooting medium
- Determine the effect of the propagation medium nutrients and the base rooting medium on rooting

Objective 3

Test rooting protocols on selected rootstocks for *in vitro* rooting

Once the callus production in an optimal hormone concentration is defined:

- **IBA dip and plant on PGR-free medium**
- **NAA dip and plant on PGR-free medium**

In Vitro Rooting Scheme

Propagation Medium	Murashige and Skoog (MS)			Pear Rootstock Medium (PRS)		
Rooting Medium	MS		PRS	MS		PRS
Rooting Hormone						
IBA (10 mM)	X		X	X		X
NAA (10 mM)	X		X	X		X

Objective 4

Test direct rooting to soil-free medium

- Test for rooting after growth on each formulation
- Test IBA and NAA dips
- These studies will take place at North American Plants, McMinnville, OR

Objective 5

Transfer protocols to commercial micropropagation facilities

- Use the combined growth medium and rooting data to develop individual protocols for rootstocks of interest.
- Transfer these protocols to micropropagation companies for use in providing rootstocks for nurseries and growers.
- No patent or licensing restrictions.



Thank you