FINAL PROJECT REPORT

Project Title: Improved micropropagation of dwarfing pear rootstocks

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Other funding sources
Agency Name: California Pear Commission
Amt. awarded: $36,900
Notes: Improved Media for Micropropagation of Dwarfing Pear Rootstocks (to supplement FPC/PPC funding for this project; for post doctoral researcher)

Total Project Funding: $35,000

Budget History:

Item	2011
Salaries	20,000
Benefits	12,000
Wages	
Benefits	
Equipment	
Supplies	2500
Travel	500
Miscellaneous	
Total	$35,000

RECAP ORIGINAL OBJECTIVES (for original 3 year project)

This was designed as a 3 yr project and funded for 1 year.

1) Develop growth medium suitable for commercial micropropagation of dwarfing pear rootstock selections and cultivars. **Year 2011-12** Spring-Summer: Multiply stocks of available cultures for testing. Initiate cultures of additional dwarfing pear rootstock cultivars or selections from grafted or forced shoots. Summer-Winter: Initial test of available cultures for "mesos" elements ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). Winter: Data analysis and preliminary report. Continue optimization studies in years 2 and 3.
2) Determine rooting potential of shoot cultures on new medium formulations (yr 2).
3) Finalize standard micropropagation and rooting protocols and transfer this information to commercial micropropagation facilities (yr 3).

SIGNIFICANT FINDINGS

- Initial growth on our improved medium allowed for enough propagation to start the experiments.
- Quality of the micropropagated shoots improved significantly for all eight genotypes with 1.5X or greater mesos ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) compared to standard MS medium**.**
- Leaf spot and edge burn symptoms, hyperhydricity and leaf curl decreased with 1.5 or 2.0X mesos.
- In most cases shoot multiplication was only slightly influenced by mesos.
- Shoot length, leaf color and leaf size were best on mesos of 1.5X or greater.

RESULTS & DISCUSSION

We initiated shoot cultures of dwarfing pear rootstocks and multiplied them for a study of the effect of MS mesos concentrations ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). Five of the eight genotypes were growing very poorly at the beginning of the experiment (OHxF69, OPR125, G28.120, Fox11 and Pyro 2-33) while the others were growing sub optimally. Increased mesos were required for moderate to good growth of all eight genotypes (Fig. 1). A range of MS medium mesos concentrations from 1.5X to 2.5 X gave the best "quality" ratings, the longest shoots, and the best leaf form and color for most genotypes (Fig. 2) but most still are very short and are not multiplying as rapidly as would be preferred. Additional genotypes were multiplied for future testing.

Quality: All eight genotypes had the best quality on mesos 1.5X to 2.5X although these were not always high ratings.

Shoot number: There were no significant differences in multiplication (this is often governed by nitrogen ratios).

Shoot length: In many cases all treatments were similar. For others (OHxF 97, Pyrodwarf and Pyro2-33) the higher mesos produced the longest shoots. Nitrogen ratios are known to affect shoot length.

Leaf color rating: Leaf color was darkest at 2.0 and 2.5X mesos. Red or yellow leaves were noted for 0.5 and 1.0 mesos plants.

Leaf size rating: Leaf size was moderate at 1.5X mesos.

Callus: Callus was not a serious problem on any of the cultivars.

The lowest mesos concentration (0.5X) gave an indication of true deficiency symptoms. In all cases the plants were stunted, with fat stems, pale in color and had reddened or spotted and curled leaves. The normal MS mesos (1.0X) plants were also small and many had leaf spotting or edge discoloration. At 1.5X shoots were slightly taller and leaves were a normal color and size. Plants on the 2.0X and 2.5X concentrations were darker green, with larger leaves and often longer stems. This experiment indicates that the rootstock cultivars and selections have a requirement for higher concentrations of 'mesos' (2.0 and 2.5X) than did the scion cultivars (1.5 and 2.0X). We will grow these selections for additional passages on the higher concentrations to determine if they are suitable for long-term propagation. This initial test shows that changes in mineral nutrition result in significant improvements in the shoots (as well as eliminating a number of the common physiological abnormalities) of the dwarfing pear rootstocks. However, most genotypes still require further improvements in shoot multiplication and shoot length (Fig. 3). Optimization of nitrogen and minor elements is needed to optimize growth and produce one or more improved growth media for use in commercial micropropagation, as well as protocols for the improvement of specific genotypes. Once commercial micropropagation is possible, these and newly introduced rootstocks would be more widely available for field testing and grower use.

A preliminary rooting test with two genotypes (OH x F 87 and Horner 51) produced 50-100% rooting for some treatments and all *in-vitro* rooted shoots survived under mist in the greenhouse. This preliminary test provided information that will be useful when designing the final rooting studies. The base medium used for multiplication and the base rooting medium both influenced the percent shoots that rooted. This will be further tested in additional studies.

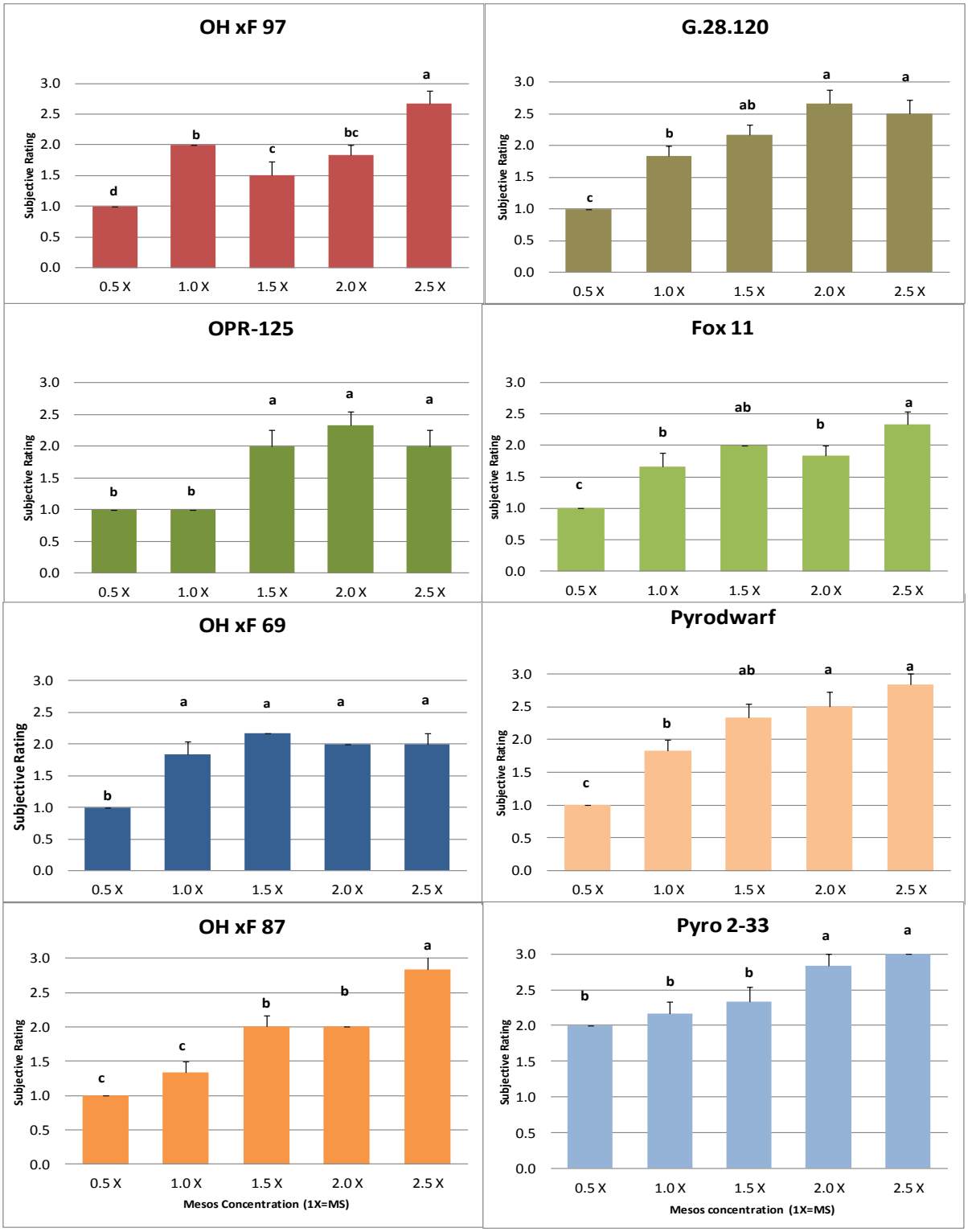

Figure 1. Mean quality ratings of shoots of eight pear rootstocks grown on MS medium with increasing concentrations of 'mesos' elements. Plants were rated for quality: 1 poor, 2 moderate, 3 good. Treatments with the same letter are not significantly different (α=0.05). n=6 shoots per treatment.

Fig. 2. Photographs of the pear shoots grown on five mesos concentrations. From left: 0.5X, 1.0X (MS), 1.5X, 2.0X, 2.5X mesos. Scale is in centimeters**.** The ideal plant would be 7-9 cm tall.

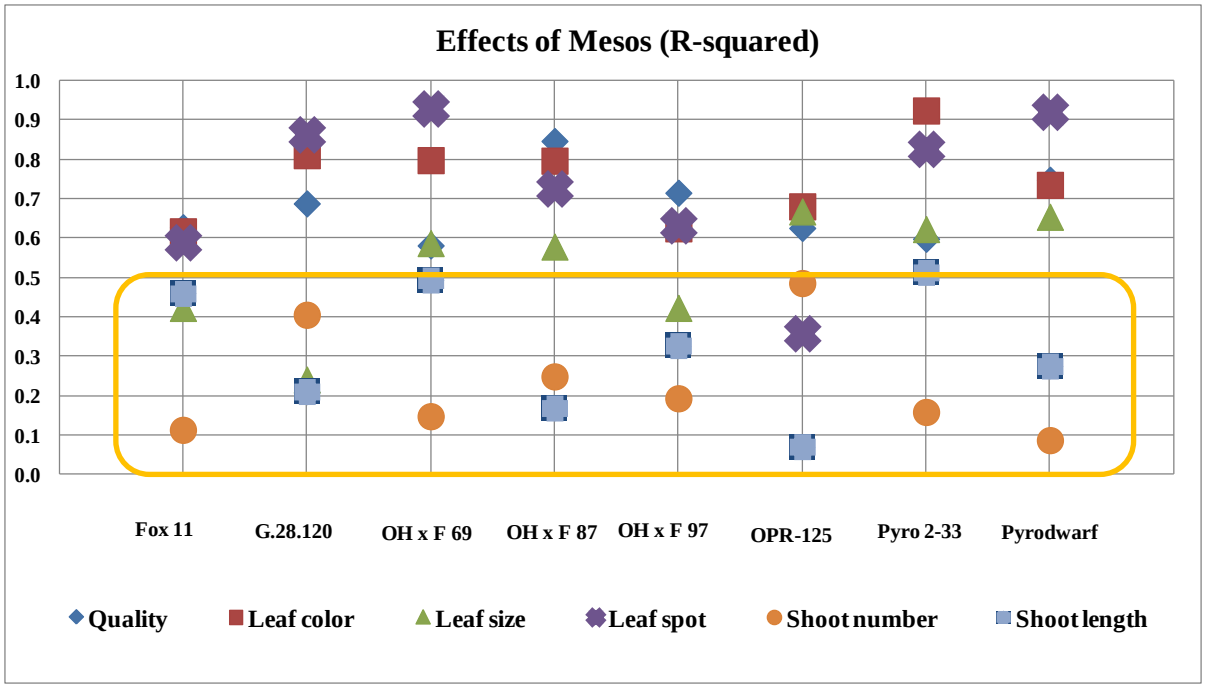

Fig. 3. This chart shows the statistical significance of the plant responses to increased mesos concentrations. Improvements resulting from increasing mesos are indicated by the top half of the chart (>0.5) while responses that did not improve are in the yellow box at the bottom. Shoot number (orange dots) and shoot length (blue square) still need improvement on most or all of the genotypes.

EXECUTIVE SUMMARY

The development and use of pear dwarfing rootstocks has been restricted by the lack of effective and rapid propagation systems. Dwarfing rootstocks are difficult to propagate both traditionally and *in vitro*. Many promising dwarfing rootstocks were abandoned because of difficulty with traditional propagation or poor growth *in vitro* (Proebsting, WTFRC reports 2003-7). Our laboratory conducted intensive studies of the mineral nutrition of *in-vitro* grown pear scion cultivars and species over the last four years. During this process we determined key nutrients in the growth medium that promote the growth of a range of cultivars and species that originally would not grow or grew poorly and slowly on standard medium (Reed et al., 2011b). Amazing improvements were seen in the 17 pears studied in these experiments. Initially most were in poor condition, but now all are showing excellent growth and multiplication with these mineral nutrient improvements. These earlier studies of scion pear cultivars indicated that the mineral nutrition factors with the most effect on plant appearance and growth were in the 'mesos' stock solutions ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$). In the current study we tested the 'mesos' concentration in medium for eight dwarfing pear rootstocks. Growth of all eight genotypes improved significantly (from poor to moderate or good) with increased 'mesos'. The best quality shoots, the longest shoots and the best leaf form and color were obtained with increased 'mesos' concentrations. Half of the tested plants were rated as good quality (rated>2 out of 3) on one of the higher 'mesos' concentrations. All the genotypes were greatly improved but are not yet of a quality high enough for routine micropropagation. Shoot number and shoot length are still subpar. Continued study of the effect of mineral nutrients, especially nitrogen and micronutrients, should result in medium formulations that will provide optimal micropropagation for all 15 genotypes in the test group. Development of growth media that can be transferred to commercial nurseries for production of the rootstocks will allow wider use of more rootstock types. Testing the most effective rooting treatments with shoots grown on improved medium formulations would also provide standard protocols for use by commercial micropropagation laboratories.