IMPROVED MICROPROPAGATION AND ROOTING OF DWARFING PEAR ROOTSTOCKS

Barbara M. Reed

ABSTRACT

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). Over the last four years our laboratory conducted intensive studies of the mineral nutrition of *in-vitro* grown pear scion cultivars and species. During this process we determined key mineral nutrients in the growth medium that promote the growth of a range of cultivars and species that originally would not grow or grew slowly on standard medium (Reed et al., 2010). Initially most of these 17 scion pear cultivars were in poor condition, but now all are showing excellent growth and multiplication with these mineral nutrient improvements. These earlier studies 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 improved significantly (from very poor or 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 on one of the higher concentrations. All the genotypes were greatly improved but they are not yet of a quality high enough for routine micropropagation. Continued study of the effect of mineral nutrients, including nitrogen and micronutrients, should follow to produce medium formulations that will provide good micropropagation for all the genotypes. This will provide growth media that can be transferred to commercial nurseries for production of the rootstocks. Testing the most effective rooting treatments with shoots grown on improved medium formulations would also provide standard protocols for use by commercial micropropagation laboratories.

OBJECTIVES

1) Develop growth medium suitable for commercial micropropagation of dwarfing pear rootstock selections and cultivars (yrs 1-2).
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).

PROCEDURES

Multiplication: Plantlets were initially grown in tissue culture boxes with 40 ml of medium per box. One of our improved pear media (Pear #1 or Pear #2)(Reed et al., 2011) were used for the initial multiplication with 1 mg/L N^6-benzyladenine (BA) under normal growth conditions and shoots were transferred to new medium every 3 weeks until enough were available for experimentation. We obtained needed cultures and multiplied them for experimental use using a medium formulation developed for scion cultivars. The eight rootstocks in Table 1 multiplied well enough to use for the initial testing.

Mesos stock concentrations: Experiments to optimize the medium for the desired cultivars were determined by the results of the initial experiments. The main driving factor in our earlier studies of pear cultivars was the mesos stock ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) so that was the first factor to test. The mesos ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) stock solution was tested at 5 concentrations (0.5 to 2.5 X of the MS concentration).

Table 1. Rootstock genotypes tested on five MS mesos concentrations.

Local number	Name
2707.001	Fox 11
2144.001	G.28.120 (P-2363)
1343.004	OH x F 69*
1345.002	OH x F 87*
726.002	OH x F 97
1314.002	OPR-125
2598.002	Pyrodwarf
2699.002	Pyro 2/33*

* High priority cultivars

Data: Data taken included shoot number, shoot height, leaf size and color, shoot tip necrosis, number of nodes per shoot and overall quality (based on industry standards). From these responses we will determine mineral nutrient formulations that result in optimal individual responses and the best overall growth.

REFERENCES

Reed, B.M., J.S. DeNoma, S. Wada, and J.D. Postman. 2011. Micropropagation of pear (*Pyrus* sp). In: M. Lambardi, E.A. Ozudogru, and S.M. Jain (eds.). Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. Humana Press-Springer, NY.

Reed, B.M., S. Wada, J. DeNoma, T.J. Evans, and R.P. Niedz. 2010. Improving *in vitro* mineral nutrition for diverse pear germplasm In Vitro Cell. Dev. Biol. 46: S64.

RESULTS & DISCUSSION

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 ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) were required for moderate to good growth of all eight genotypes (Figure 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 (Figure 2).

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.

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

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

The lowest mesos concentration (0.5X) gave an indication of what deficiency symptoms are expressed *in vitro*. In all cases the plants were stunted, pale in color and had reddened or spotted and curled leaves. The normal MS mesos (1.0X) plants were also small and many had spotting or leaf discoloration although not as extreme as the lower level. 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 preliminary 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 improvements in mineral nutrition make significant improvements in growth (as well as to eliminating a number of the common physiological abnormalities) of the dwarfing pear rootstocks. Completing the study would provide improved growth medium for use in commercial micropropagation. Once commercial micropropagation is possible, the rootstocks would be more widely available for field testing and grower use.

- 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.
- Shoot length, leaf color and leaf size were best on mesos of 1.5X or greater.

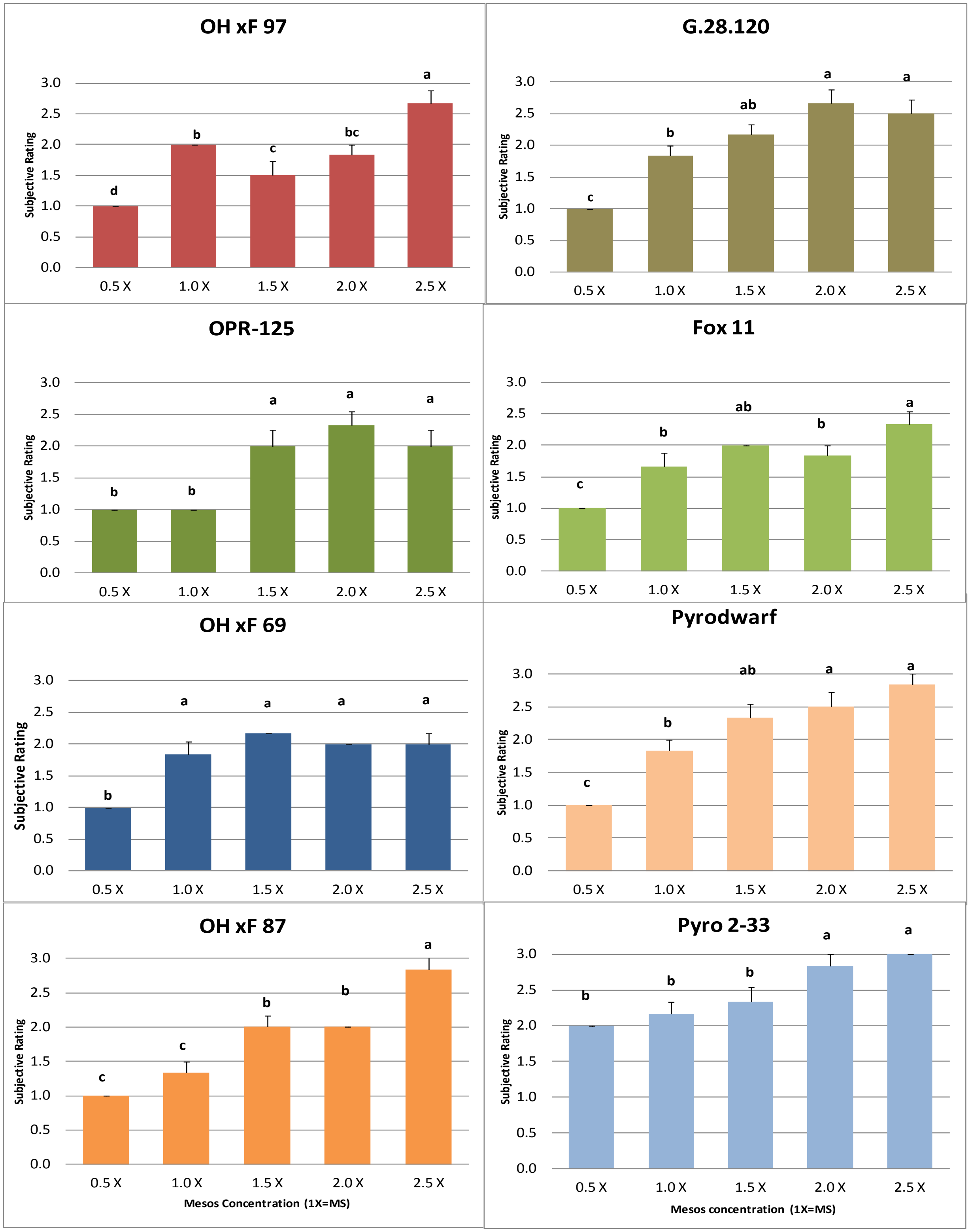

Figure 1. Mean quality ratings of shoots of eight pear rootstocks grown on MS medium with increasing concentrations of 'mesos' elements. Plants were rated for quality: 1 poor, 2 moderate, 3 good. Treatments with the same letter are not significantly different (α=0.05). n=6 shoots per treatment.

Figure 2. Photographs of the pear shoots grown on five mesos concentrations. From left: 0.5X, 1X, 1.5X, 2X, 2.5X mesos. Scale is in centimeters**.**