PROJECT REPORT **YEAR**: **2**

IMPROVED MICROPROPAGATION AND ROOTING OF DWARFING PEAR ROOTSTOCKS

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ABSTRACT

The development and use of dwarfing pear rootstocks is greatly restricted by the lack of effective and rapid propagation systems. Dwarfing rootstocks are difficult to propagate both traditionally and *in vitro*. Improving the *in vitro* propagation (micropropagation) of a range of dwarfing rootstocks would provide materials for field testing, and eventually for commercial propagation and use by growers. *In vitro* culture is often used to rapidly produce large numbers of plants of new cultivars; however not all cultivars will grow well on any of the standard growth media. We applied a computer based methodology to the mineral nutrition of pear, since many cultivars and species are difficult to grow in tissue culture. Initially we found that the $CaCl_2$, $MgSO_4$, KH_2PO_4 (mesos) and nitrogen components of Murashige and Skoog (MS) growth medium were very important to shoot growth. The current study found improved shoot quality for 10 rootstock selections grown on the highest mesos concentration (2.5X the normal level). This is much higher than the 1.5 to 2X mesos that was optimum for scion cultivars in our earlier study. Using this 2.5X concentration as the base level for testing nitrogen concentrations we found four patterns of nitrogen usage by the 10 rootstocks tested. By increasing the mesos concentration to 2.5X, the normal MS nitrogen ratios were better utilized by eight of the genotypes resulting in much improved growth over the MS formulation. The optimum concentrations of ammonium and nitrate nitrogen could be optimized for each of the 10 selections to produce high quality micropropagated shoots.

OBJECTIVES:

1) Develop growth media suitable for commercial micropropagation of dwarfing pear rootstock selections and cultivars.
2) Determine rooting potential of shoot cultures on new medium formulations.
3) Determine standard micropropagation, rooting and acclimation protocols and transfer this information to commercial micropropagation facilities.

PROCEDURES:

Multiplication for the stock materials. Plantlets of 10 pear rootstock selections (Table 1) were cultured in tissue culture boxes with 40 ml of medium per box. Our improved pear medium (1.5X mesos) as determined from the last experiments was used with 1 mg/L N^6-benzyladenine (BA) under normal growth conditions. Shoots were transferred to new medium every 4 weeks (rootstocks are slow growing and required a week longer than scion cultivars) until enough are available for experimentation.

Table 1. Dwarf/semi dwarf rootstock selections used for nutrition testing *in vitro*.

Identifying number	Plant name	Species
2707.001	Fox 11	*P. communis* Italy IPR
2144.001	G.28.120 (P2363)	Brossier rootstock France IPR
2955.001	Horner 4	*P. communis*
2956.001	Horner 10	*P. communis*
1572.001	OH x F 333	*P. communis*
1314.002	OPR 125	*P. calleryana*
1170.002	OPR 157	*P. calleryana*
1379.001	OPR 260	*P. betulifolia*
2699.003	Pyro 2-33 (Rhenus 3)	*P. communis* Germany IPR
2598.002	Pyrodwarf	*P. communis* Germany IPR

Testing of medium formulations. Shoots were grown for three 4-week passages on each new medium formulation and standard MS medium was used as the control. Multiple passages (at least 3 times for 4 weeks subculture cycle) are needed to stabilize the plant growth for accurate evaluation.

Mesos concentration. The 10 selections were grown on three mesos ($CaCl_2$, $MgSO_4$, KH_2PO_4) concentrations (1.5X, 2.0X and 2.5X MS). This initial test indicated that the dwarf and semi dwarf pear rootstocks grow best with the highest mesos (2.5X).

Nitrogen ratio. The second propagation test with nitrogen compounds was performed to determine the ratios of ammonium and nitrate (Table 2) for the optimal growth and development for dwarf and semi-dwarf rootstocks using the optimized meso salts (2.5X MS) as the base medium.

Data. Data taken for all experiments 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. Six of the resulting shoots from each treatment were used for data collection and four were photographed.

Table 2. Eight concentrations of ammonium (Am)/ potassium (K) / nitrates (N) tested.

	Treatment (T)	NH_4 (mM)	K (mM)	NO_3 (mM)	Mesos (x)
T1	Med Am / K/ N)	20	20	40	1.0 (MS control)
T2	Low Am / K / N	5	15	20	2.5
T3	Med Am / K/ N	20	20	40	2.5
T4	Low Am/ High K/ Med N	10	30	40	2.5
T5	Med Am/ Low K/ Med N	30	10	40	2.5
T6	High Am/ K/ N	30	30	60	2.5
T7	Low Am/ High K/ High N	15	45	60	2.5
T8	High Am/ Low K/ High N	45	15	60	2.5

RESULTS AND DISCUSSION

Shoots of all ten rootstocks grown on the highest mesos ($CaCl_2$, $MgSO_4$, KH_2PO_4) concentration (2.5X MS) produced the best quality (Fig.1). This is much higher than the 1.5 to 2X that was optimum for scion cultivars in our earlier study. While the 1.5X concentration reduced the deficiency symptoms seen on standard MS medium (data not shown), greatly increased shoot elongation and multiplication were noted with higher mesos concentrations. Only 'Pyrodwarf', the most extremely dwarfing rootstock, remained small with the 2.5X treatment. The 2.5X mesos concentration was then used for the nitrogen ratio testing.

Seven nitrogen combinations were tested with the 2.5X mesos concentration and the standard MS medium was used as a control medium (Table 2). Visual observation showed that nine of the 10 rootstocks had improved growth on 2.5X MS with one or more of the nitrogen combinations and examples are shown in Fig. 2. Eight of the 10 genotypes grown on T3 (MS nitrogen with 2.5X mesos) showed improved quality when compared to the standard MS formulation (T1)(Fig. 2). Overall, four patterns of nitrogen use were found when the data was analyzed with software designed to project the best possible quality. Six rootstock genotypes (three *P. communis* Fox 11, Horner 10, Pyro 233; two *P. betulifolia* OPR 113, OPR260; and *P. calleryana* OPR125 had the best projected growth on moderate to low NO_3/ high NH_4/and low K (Fig. 3A). Horner 4 (*P. communis*) and G28.120 Brossier (*P. nivalis*) required equal NH_4 and K concentrations regardless of NO_3 (Fig. 3-B). OHxF333 (*P. communis*) grew best with low NH_4 and high K regardless NO_3 (Fig. 3C). Pyrodwarf (*P. communis*) was not significantly better overall, however the trend was that better growth was on low NO_3 regardless of NH_4 and K (Fig. 3D). Although we have not yet grown these plants on the projected "best" regions, plants grown on several of the design points gave excellent results and could be used to commercially propagate these rootstock selections. Because these rootstocks are the result of complex breeding, or are species other than *P. communis,* it is logical that there would be some diversity of response to the nitrogen ratios. The dwarfing characteristics of these rootstocks might also be a result of their ability to use nitrogen in the forms normally available in the soil. Six of the cultivars were best at moderate to low NO_3/high NH_4/and low K (Fig. 3A) that has more NH_4 than MS. The models projected for the three *P. communis* and the *P. nivalis* Brossier rootstocks (Fig. 3B, C, D) indicate that they could have even better growth when grown 2.5X mesos but also on nitrogen ratios quite different from MS.

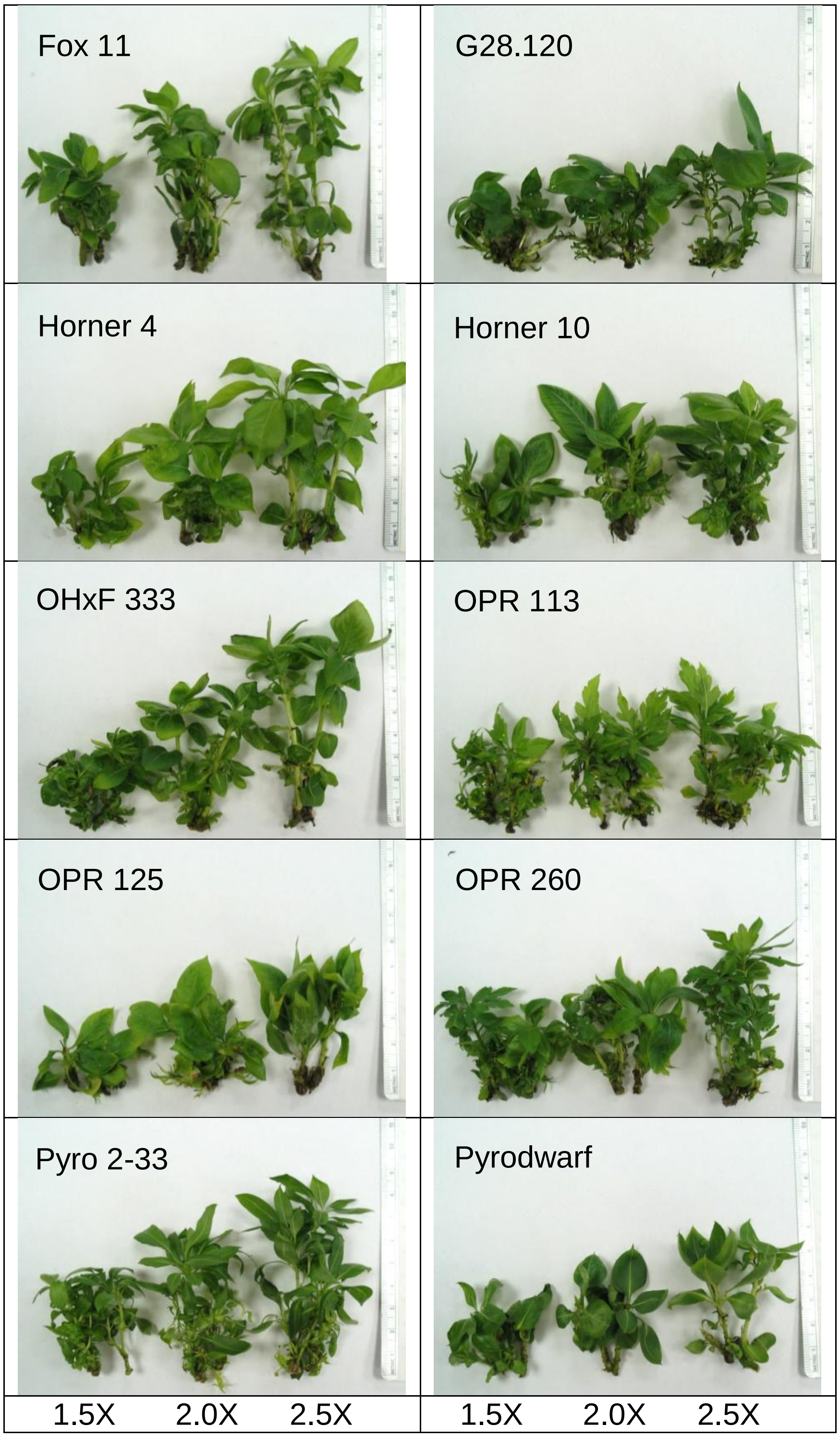

Figure 1. Ten rootstock cultivars grown on three mesos concentrations


G28.120
T1
T2
T3
T4
T5
T6
T7
T8
Horner 10
T1
T2
T3
T4
T5
T6
T7
T8

Fig. 2. Photos of rootstock grown on 8 combinations of nitrogen with high mesos media (Table 2) (T1: MS control)

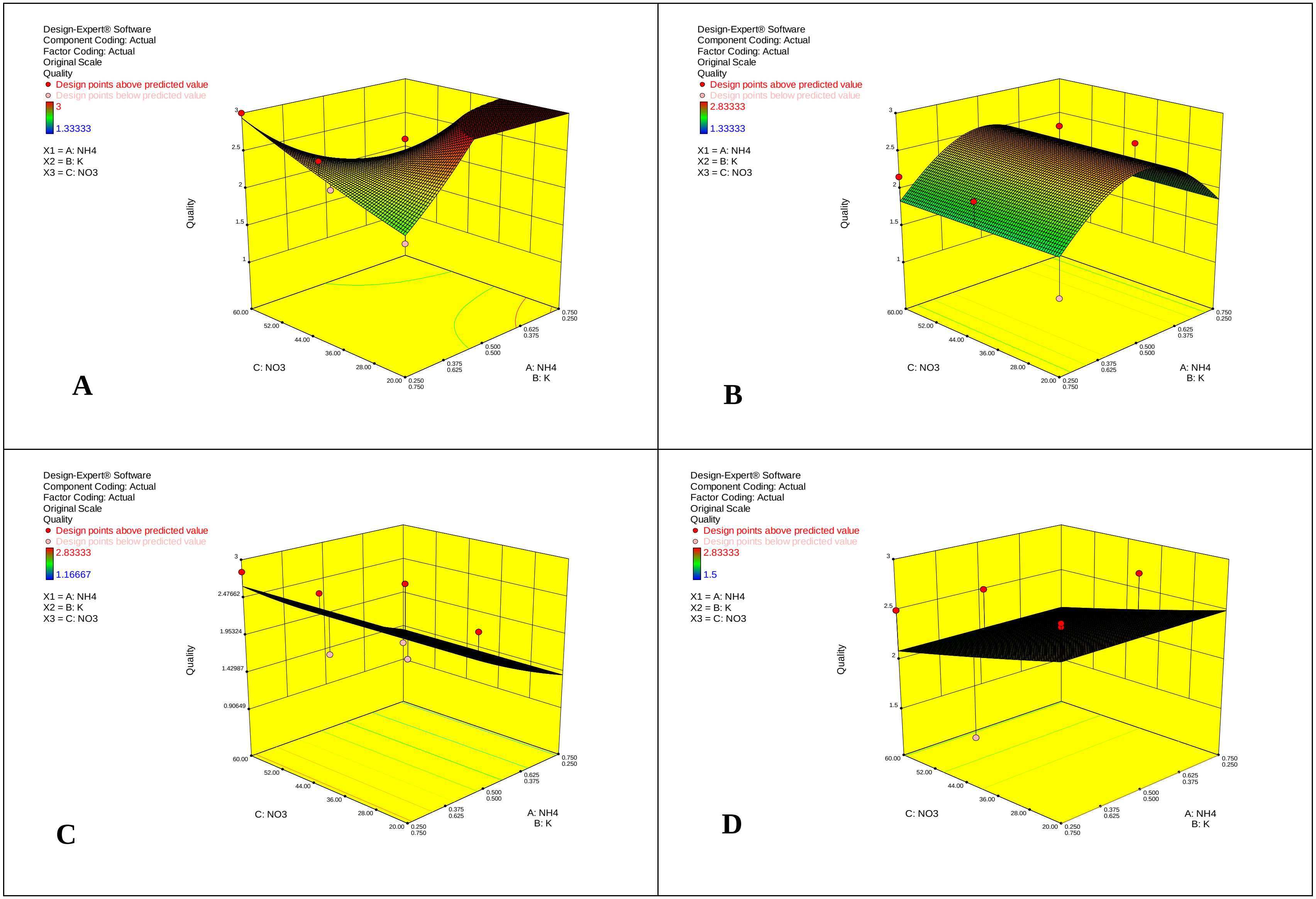

Fig. 3. Projected areas of best growth on 2.5X mesos media for A) six genotypes (three *P. communis* Fox 11, Horner 10, Pyro 233; two *P. betulifolia* OPR 113, OPR260; and *P. calleryana* OPR125 on a range of nitrogen ratios. B) *P. nivalis* G28.120 and *P. communis* Horner 4. C) OHxF 333 (*P. communis).* D) Pyrodwarf (*P. communis*).