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Project Title: Improved Mineral Nutrition for Micropropagation of Woody Nursery Crops

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Objective: Determine the effect of mineral nutrition on plant appearance, shoot initiation, and elongation of pear. From these responses we will determine mineral nutrient formulations that result in optimal individual responses and the best overall growth. Data taken will include shoot length, shoot multiplication, number of nodes per shoot, and a subjective rating of plant appearance (based on industry standards). We will finish with a study on the effect of these improved growth formulations on rooting.

Summary: We determined from initial tests that the factor with the most effect on plant appearance and growth was the 'mesos' stock ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) followed by the iron and ammonium components. Mesos stock was optimized with two experiments and it was determined that all the pear genotypes required 1.5X to 2.0X the amount of mesos compared to what is found in MS medium. Some physiological abnormalities were noted. We were able to divide the genotypes into two groups based on the "mesos" response. Although the individual factor ($CaCl_2.2H_2O$, KH_2PO_4, $MgSO_4$) response varied with the genotype and with the characteristic measured, good results could be obtained by simply increasing all three as a group. Tests of iron found the standard MS concentration to be good for all genotypes with higher and lower concentrations inhibiting growth.

Nitrogen was a significant factor for plant growth and a study was done to test variations of the ammonium/nitrate ratios. The 16 genotypes were grown on the best mesos concentration (1.5 or 2.0X) with the 7 treatments or on a standard MS medium control. All of the *P. communis* and most of the other species had the best quality on the improved mesos medium (1.5 or 2.0X mesos) with the

standard MS nitrogen (20 NH_4 / 20 K /40 mM NO_3) and the growth was significantly better than on the control MS medium with the standard 1.0X mesos and 1.0X nitrogen. This indicates that improving the 'mesos' stock ($CaCl_2.2H_2O$, KH_2PO_4, and $MgSO_4$) made changes in the nitrogen unnecessary for most genotypes. In individual cases increased ammonium produced better shoots. Shoot multiplication results were quite different from the quality levels. For the 1.5X meso pears, shoot number was best (3.5-6.5 shoots per plant) with lower ammonium and nitrate concentrations. The 2.0X meso pears produced fewer shoots overall (~2.5 shoots per plant) than the 1.5X meso plants, but for most the standard MS ratios were the best. With these lower multiplication rates, it may be preferable to multiply all the plants on the 1.5X mesos with lower ammonium and "finish" the final shoots on regular MS nitrogen.

Initial rooting studies found that rooting was very genotype dependent (Fig. 1). Horner 51and OHxF 87 rooted vigorously (50 to 100%) (Fig. 2), while Hang Pa Li, Harbin, and *P. pyrifolia* (*P. pyrifolia* and Sion Sz Mi) had little or no rooting (Table 1). *P. cordata* produced roots on 16% and *P. koehnei* on 33% of shoots. The *P. pyrifolia* accessions exhibited high sensitivity to the IBA treatment and several died. None of the controls produced roots. Ex-vitro rooting of the non-rooted shoots did not produce roots or root hairs by 4 weeks (Fig. 3), suggesting that ex-vitro rooting with Rootone treatment may require longer time periods than 4 weeks or may not be effective. Additional study is needed to determine rooting procedures for species other than *P. communis*.

Table 1. *In vitro* rooting responses of eight pear genotypes to a 15 sec dip in 10 mM IBA.

TC media	Hang Pa Li	Horner 51	*P. koehnei*	*P. pyrifolia*
1.0 x Control	0*	0	0	0
1.5 x Control	0	0	0	0
1.0 to 1.0 x	0	6	0	1
1.0 to 1.5 x	0	3	1	1
1.5 to 1.0 x	1	2	2	0
1.5 to 1.5 x	0	3	0	0
TC media	Harbin	OHxF 87	*P. cordata*	Sion Sz Mi
1.0 x Control	0	0	0	0
2.0 x Control	0	0	0	0
1.0 to 1.0 x	0	6	1	0
1.0 to 2.0 x	0	5	0	0
2.0 to 1.0 x	0	6	2	1
2.0 to 2.0 x	0	3	2	0

*Rooted shoots were counted at planting. There were six shoots in each treatment (n=6). Controls were not dipped. There was no additional rooting after 4 weeks in the mist bed.

Fig. 1. Pear shoots in potting medium under mist. Green tags indicate plants that rooted *in vitro* and white tags for non-rooted plants that were dipped in Rootone before planting for *ex vitro* rooting.

Fig. 2. After 4 weeks in the mist bed the *in-vitro* rooted pear shoots grew additional vigorous roots (Left: OHxF 87, Middle: Horner 51, Right: *P. pyrifolia*).

Fig. 3. Non-rooted plants were dipped in Rootone before planting for *ex vitro* rooting. Many survived under mist for 4 weeks but did not produce roots (From left: Hang Pa Li, Horner 51, *P. koehnei*, and Sion Sz Mi).