Abstract  The USDA-ARS National Clonal Germplasm Repository in vitro collection contains over 200 pear accessions in 18 species. Due to the wide genetic diversity of this collection there is also a diverse response to growth on standard tissue culture media. An initial study of mineral nutrition using a systematic response-surface approach with five mineral stock solutions (ammonium nitrate, potassium nitrate, Mesos, minors and iron) found that the Mesos factor affected the most responses and the most genotypes. This study was designed to optimize the Mesos (CaCl₂, MgSO₄, and KH₂PO₄) components on 10 pears (Pyrus communis cv., P. calleryana, P. dimorphothyalla, P. kaehni, P. ussuriensis and P. pyrifolia). Sixteen factors were evaluated including overall quality, shoot multiplication and shoot height. Short stature, leaf spots, edge necrosis and red or yellow coloration were some of the main symptoms of poor nutrition in these diverse pears. Increased concentrations of all three constituents of the Mesos solution decreased leaf symptoms, and significantly increased overall plant quality (P<0.0001). Treatments with >1.5x the MS concentrations of all three chemicals produced the best quality ratings for all 10 genotypes. Concentrations producing the best growth varied by genotype, but all required higher than MS concentrations for the best growth and multiplication. A further test of 18 genotypes found that increasing all the components equally to 1.5 or 2.0X the MS levels produced high quality plants for all genotypes. Resulting shoot growth was healthier and taller, leaves were greener plus leaf spotting, discoloration and edge burn were eliminated.

Introduction  Mineral nutrition of in vitro plants is often difficult to optimize due to complex chemical interactions of required nutrients. Plant growth response on standard growth medium varies widely due to the wide genetic diversity of germplasm collections. Many pear cultivars and species are recognized as recalcitrant in tissue culture (Bell and Reed 2002). Preece (1995) reported that mineral nutrition critically influences plant culture morphogenesis. A systematic approach is required to efficiently evaluate the large number of components in a typical formulation such as the thirteen essential mineral nutrients in Murashige and Skoog (MS) medium (Epstein and Bloom 2005). Previous study by improved computer-designed experiments (Niedz and Evens, 2006) in our lab found that the Mesos (Ca, Mg, P) was the most influential factor in in vitro growth of 5 pear genotypes. This study was to determine the optimal concentrations of each of the Mesos compounds for pear culture.

Experimental design  
- Designed to optimize Mesos components in growth medium of diverse pear germplasm using Design Expert 8 software (Stat-Ease. MN. USA).
- Evaluated factors included a subjective rating of plant appearance, shoot length, shoot multiplication, leaf color, leaf spots/ necrosis, calyx and leaf size.
- Three plants in predetermined positions in each box were evaluated and the two remaining plants were photographed.

Materials & Methods  
- **First experiment:** 10 pear genotypes grown on 24 combinations of the three Mesos components (Ca, Mg, P) and a MS control. Experiment was performed sequentially in three blocks of 24 treatments per block with two controls (MS) included with each block.
- **Second experiment:** 5 concentrations of Mesos (0.5 to 2.5X) for additional 8 genotypes (total 18 pears)
- Five 2-node plants without apical section were grown in each of 2 tissue culture vessels (Magenta GA7).
- Stock shoot cultures were grown in 40 mL of medium and transferred to new medium every 3 weeks (3 times).
- At 25˚C under a 16 h photoperiod with 70-90 μMm-2s-1 irradiance provided by a combination of cool and warm white florescent bulbs.

**Fig. 1.** The best quality ratings were with >1.5X MS Mesos for all 10 genotypes (yellow - orange zone).
**Fig. 2.** High Mesos (>1.5X) reduced leaf spots and edge burn symptoms (blue zone).
**Fig. 3.** Ideal leaf size is medium (2-2.5). Leaf size was increased by higher Mesos concentrations.
**Fig. 4.** Shoot number increased with lower Mesos concentrations.
**Fig. 5.** Growth improvements of ‘Bartlett’ and ‘OH x F 87’ shoots on media with low to high Mesos (Pictures show the examples of poor growth from the left, optimal growth to the right, by Mesos concentration).

Summary  
- Mesos stock (CaCl₂·2H₂O; KH₂PO₄; MgSO₄) was the driving factor in pear shoot quality and many of the factors that make up healthy shoots and high plant quality.
- Shoot length and leaf size were significantly increased with increasing Mesos for most of the pear genotypes with increased phosphorous while improved leaf quality may be due to improved calcium and magnesium nutrition.
- Factors that increased shoot length tended to decrease shoot number.
- Concentrations producing the best growth varied by genotype, minimal concentrations of each 3 Mesos factor were specifically required for their best growth: CaCl₂ for Hang Pa Li, MgSO₄ for Horner S1 and KH₂PO₄ for Capital

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Reference cited  