Improved micropropagation of ornamental trees and apple rootstock

2018 Final Report

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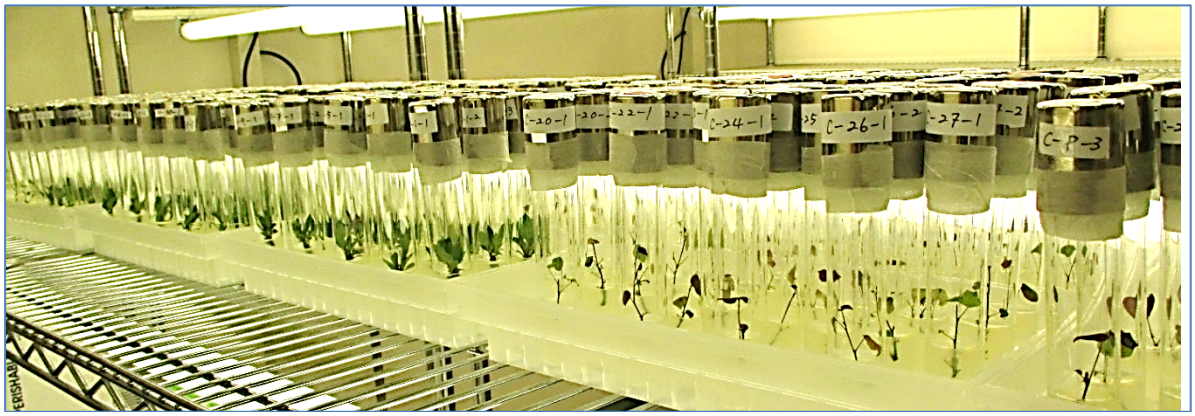
Woody plants are habitually difficult to propagate by either traditional or in vitro techniques. Frequently the most desired cultivars are the most problematic. Many ornamental tree species are produced through micropropagation, but there are wide variations in growth response among cultivars from good growth to impossible to propagate. In addition, newly developed dwarfing apple rootstocks are difficult to propagate. There is a need for improved growth media formulations to suit these diverse cultivars. Media development has typically involved testing existing formulations to find one that provides adequate growth and development but this is haphazard and time consuming. In this study we determined mineral nutrient requirements as well as carbon source, iron, plant growth regulators and antioxidants for Kentucky Coffeetree, *Gymnocladus dioicus* 'Espresso', Eastern Redbud *Cercis canadensis* 'Forest pansy', and the apple rootstock 'Geneva 214'.

Initial Study 2017: None of these cultivars grew well in culture as seen for the original stock plants on MS medium (Fig. 1). The cultures were tested with variations of the five major stock solutions using a response surface methodology and computer modeling.

Fig. 1. Tubes of shoots of the three genotypes on the initial 5 factor mineral nutrient screening in combinations of the initial 34 treatments for each genotype.



The initial experimental design was modified to allow for the use of fewer treatments and fewer shoots per treatment because the initial shoots were growing very poorly. The five factor mineral nutrient requirements for each genotype were analyzed with Design Expert software. These results are tentative because the stock plants were in poor condition and some treatments died due to this. A tentative common recipe was formulated from this initial testing for use in additional tests. This initial recipe provided better growth and reduced physiological symptoms for the stock cultures of all genotypes compared to the original (1x) MS medium: 0.5x NH_4NO_3, 0.5x $Ca(NO_3)_2$, 0.5x $MgSO_4$, 1.0x $CaCl_2$ and 2.75x KH_2PO_4.

<u>2018 Study:</u>

Objectives

Each species will have a customized recipe at the end of the experimental sequence, but this tentative recipe allowed propagation for additional experiments. These studies will also give us a better idea of the general requirements for other members of each genus studied.

Methods and timelines

Culture and Conditions. The four cultivars will be grown on trial media selected from the first studies. This is MS medium modified with 0.5x NH_4NO_3, 0.5x $Ca(NO_3)_2$, 0.5x $MgSO_4$, 0.5x $CaCl_2$ (2.5x for *Cercis*) and 2.75x KH_2PO_4 and per liter: 30 g sucrose, 2 μM N^6benzyladenine (BA) and 0.6% (w/v) agar (PhytoTechnology Laboratories A1111). *Mineral Nutrition.* The first step will be to evaluate the four cultivars on the trial media developed in year 1. After three passages the same data will be taken and evaluated. Then a second experimental design will be developed to further improve growth with the minor nutrients. Since these are diverse species, this will be a separate design for each cultivar based on the first study. Plants will be examined for shoot quality, shoot length, number of shoots, leaf size and color and disorders. *Statistical Analysis.* The mean plant responses from the nine plants of each genotype grown in the same treatment for 10 weeks will be used for analysis. The data will be analyzed using Design-Expert 8 (Design Expert, 2010) or SAS.

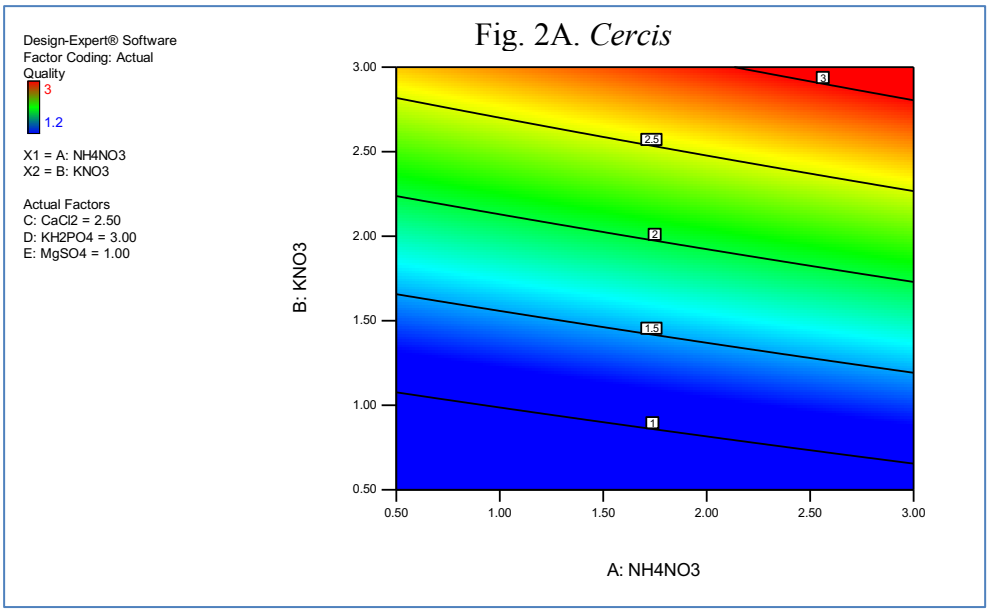
Timeline:

Winter-Summer 2018: Grow cultivars on test media from the first experiments 3 times for 4 week intervals. Take data at 16 weeks.

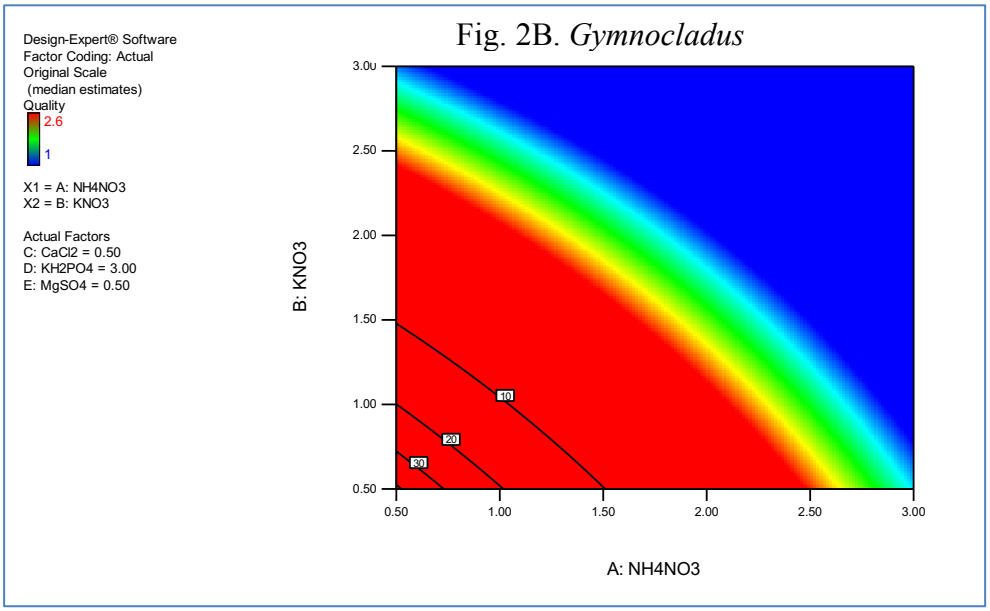
Fall-winter 2018: Factors will be tested 3 times for 4 week intervals on the test medium. Take data at 16 weeks. This may need to be completed in 2019.

Winter 2018: Data analysis and final report on mineral nutrition tests

Fig. 2. Growth requirements for the three species based on initial experiment. This model was based on 34 treatments and analyzed using Design Expert software. Red area indicates best quality shoots and projects the optimal directions.



***A. Cercis*:** Requires high amounts of nitrogen, Ca and KH_2PO_4.



***B. Gymnocladus*:** Required high amounts of KH_2PO_4, normal nitrogen and low amounts of all other nutrients

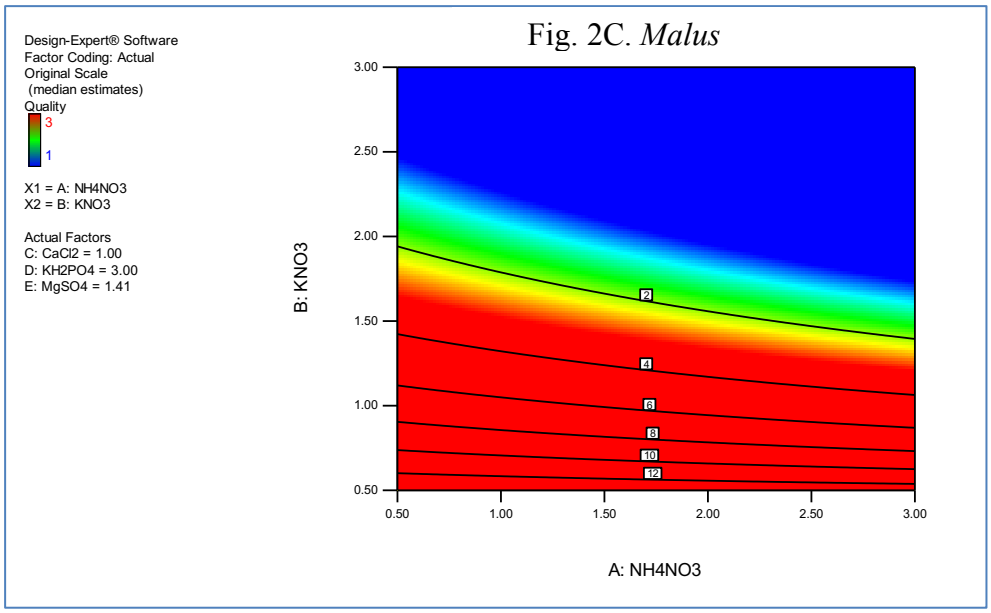


Fig. 2C. *Malus*

***C. Malus*:** Requires high amounts of KH_2PO_4, 1.4x MgSO4, and normal nitrogen and calcium.

After the mineral nutrient requirement screenings for the three genotypes (Fig. 2A, B, and C), additional adjustments for further growth improvements were necessary. Depending on genotype, such factors for additional testing included optimal carbon and iron (Fe) sources, amount of benzyl amino purine (BA), necessity for use of gibberellic acid (GA_3) and/or salicylic acid (SA). Fig. 4 shows the condition of the initial explants received (Fig. 4A, B, C), and shoot growth after modified nutrition formulations and additional adjustments (Fig. 4 D, E, and F).

Factors tested:

Carbon sources for sucrose, glucose and sorbitol were tested at 20, 25, 30 and 35 g/L initially. Additional tests combining glucose and sorbitol combinations were with 30 g individually and 15g/15 g combinations. Iron was tested from MS iron to chelated iron MS iron to Fe (EDDHA) at 0.2 g/L Salicylic acid at 5 -10 mg/L concentrations for two (Cercis and Gymnocladus). GA_3 at 0.05 – 0.1 mg/L concentrations. Four boxes with 16 plants (n=64) each were grown on each treatment and data was taken after three passes (12 weeks total).

Results and Discussions:

1. *Cercis canadensis* 'Forest pansy'

Based on our studies, the carbon source was changed from sucrose to glucose (30 g/L) and then increased to 35 g/L. This change improved overall quality and shoot numbers. To improve plant color, the iron source was changed from MS iron to Fe (EDDHA) at 0.2 g/L. Addition of salicylic acid 50 mg/L reduced phenolics and GA_3 0.1 mg/L improved shoot height (Table 1; Fig. 3, 4).

2. *Gymnocladus dioicus* 'Espresso'

Study of the carbon source indicated sucrose is not an optimal, with a change from sucrose to glucose 30 g/L and then and an increase to 35 g/L. The iron source was changed from MS Fe to

Fe (EDDHA) at 0.2 g/L for improved plant color significantly. Additional studies indicated the requirements for added SA 50 mg/L and GA_3 0.1 mg/L (Table 1; Fig. 4).

3. 'Geneva 214'

The apple rootstock had very different requirements from the ornamental legumes. To improve shoot elongation and reduce tiny multi shoots at bases for this cultivar, the BA was reduced from 1 mg/L to 0.5 mg/L and GA_3 0.1 mg/L was added. The carbon source was initially changed from sucrose to glucose 30g/L but this resulted in increased phenolic exudation so sorbitol was tested. Sorbitol 35g/L improved growth, but additional tests indicated that a mixture of sorbitol 15 g/L and glucose 15 g/L was much better for healthy growth (Table 1; Fig. 4).

Table 1. Medium formulations of MS medium and new media for *Cercis*, *Gymnocladus* and *Malus* shoot cultures.

Stock solutions amount /L MS medium	Standard MS	*Cercis*	*Gymnocladus*	*Malus*
NH_4NO_3 82.5 g	10 ml/L	10 ml/L	10 ml/L	10 ml/L
KNO_3 95 g	10 ml/L	20 – 30 ml/L	10 – 20 ml/L	10 ml/L
$CaCl_2$ 44 g	10 ml/L	25 ml/L	25 ml/L - 5	10 – 25 ml/L
KH_2PO^4 17 g	10 ml/L	30 ml/L	30 ml/L	30 ml/L
$MgSO_4$ 37 g	10 ml/L	10 ml/L	10 ml/L -5	10 – 15 ml/L
Sulfates $MnSO_4$: 1.66 g $CuSO_4$: 0.0025 g $ZnSO_4$: 0.86 g	10 ml/L	10 ml/L	10 ml/L	10 ml/L
Micros KI: 0.083 g/L $CoCl_2$: 0.0025 g/L H_3BO_3: 0.86 g/L NaMO: 0.025 g/L	10 ml/L	10 ml/L	10 ml/L	10 ml/L
Iron + EDTA	10 ml	0.2 g/L FE EDDHA Sequestrene iron	0.2 g/L FE EDDHA Sequestrene iron	10 ml
MS Thiamine (100 mg/L)	MS vitamins	50 ml/L	25 ml/L	25 ml/L
Benzyladenine stock (100 mg/L)	10 ml/L	15 ml/L	10 ml/L	5 ml/L
GA_3	--	0.1 mg/L	0.1 mg/L	0.1 mg/L
Inositol	250 mg/L	250 mg/L	250 mg/L	250 mg/L
Salicylic acid	--	50 mg/L and	50 mg/L	--
Carbon sources	30 g/L Sucrose	30 g/L Glucose	35 g/L Glucose	Sorbitol –15 g/L Glucose –15 g/L
pH adjusted		5.7	5.7	5.7

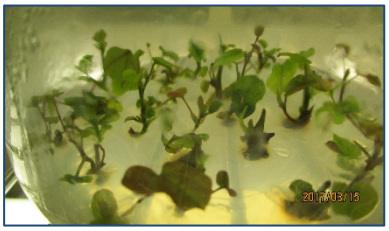
Agar (use only Phyto Tech. A1111)		6.5 g/L	6.5 g/L	6.5 g/L

Cercis canadensis 'Forest pansy' explants initially showed severe nutritional disorders on the leaves on MS medium (Fig. 3A), however, after with all modifications applied the new growth started to show beautiful and healthy dark foliage colors with some multiplications and taller statues (Fig. 3B and 3C).

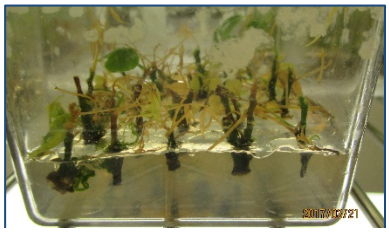
A. Initial plant form

B. Improved growth

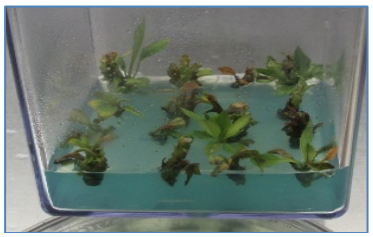
Fig. 3. Initial growth form (A) and improved growth (B) of Cercis canadensis 'Forest pansy' after medium improvements.



A. *Cercis*



B. *Gymnocladus*



C. *Malus*

D. *Cercis*

E. *Gymnocladus*

F. *Malus*

Fig. 4. Initial plant growth (A,B,C), and growth for the three species after all medium modifications (D, E, F) shown in Table 1.

Future work needed:

The *Cercis* cultivar produced excellent growth following the medium modifications. This cultivar requires no more immediate modifications. The *Gymnocladus* cultivar was improved, but still requires some modifications to improve multiplication and elongation.
The dwarf apple rootstock 'Geneva 214' grows better on this medium, however it produces phenolics when glucose is the carbon source. This requires further testing of the best combinations of carbon sources (ratios of sorbitol to glucose), and/or the use of effective absorbents such as activated charcoal (AC) should be further tested. As well as, the optimal GA_3 concentration for overcoming the short statue in the growth medium and alternative cytokinins such as changing from BA to Meta-Topline etc. should be intensely explored in the next year.