Improved micropropagation of ornamental trees and apple rootstock

2017 Final report

December 20, 2017

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Woody plants are often 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 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 initial mineral nutrient requirements for Kentucky Coffeetree, *Gymnocladus dioicus* 'Espresso', Eastern Redbud *Cercis canadensis* ' Forest pansy', and the apple rootstock' Geneva 214'.

Results

None of these cultivars grew well in culture as seen for the original stock plants on MS medium (Fig. 1), so the initial experimental design was modified to allow for the use of fewer treatments and fewer shoots per treatment. The initial study results (Fig. 2) are tentative due to this small number of shoots, however this study did produce improved growth for all three cultivars on some of the treatments (Fig. 3A, B and C), providing a starting point for further improvement.

Fig. 1A. *Cercis*

Fig. 1B. *Gymnocladus*

Fig. 1C. *Malus*

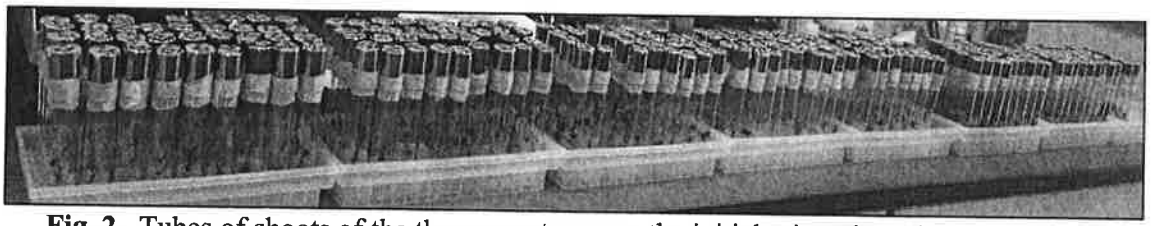

Fig. 2. Tubes of shoots of the three genotypes on the initial mineral nutrient treatments.

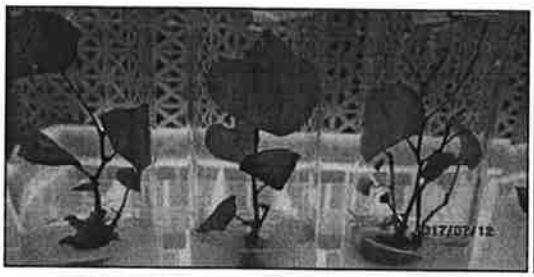

Fig. 3A. *Cercis*

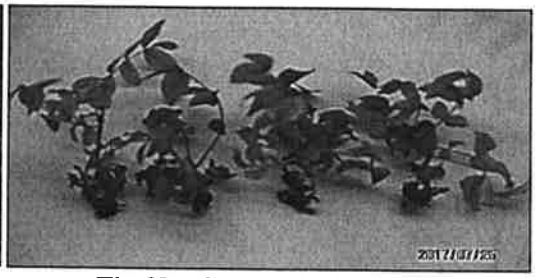

Fig 3B. *Gymnocladus*

Fig. 3C. *Malus*

A tentative recipe formulated from this initial testing, 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.

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

Mineral nutrient screening with 32 treatments (Table 1) with PRS as a comparison, produced a model indicating the nutrient requirements for each of the genotypes (Fig. 4A, 4B and 4C). This model includes all five factors tested with three to the right of the graph and the nitrogen compounds on the graph axes. The overall quality of the shoots is indicated by the graph color. Areas of the graph that are blue or green indicate shoots with poor growth while those in orange or red are good growth. *Malus* shoots had the poorest response, but were improved from growth on earlier media.

Mineral Nutrient Requirements as indicated by the model:

1. *Cercis*: Requires high amounts of Ca and KH_2PO_4 and low amounts of nitrogen and Mg
2. *Gymnocladus*: Requires high amounts of KH_2PO_4 and low amounts of all other nutrients
3. *Malus*: Requires high amounts of KH_2PO_4 and low or normal amounts of all other nutrients

Table 1. Mineral nutrient screening with 32 treatments with PRS as a comparison. Five factors were tested with concentrations ranging from 0.5x to 3x MS medium.

Table 1.	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Treatment	NH_4NO_3	KNO_3	$CaCl_2$	KH_2PO_4	$MgSO_4$
1	1.21	3.00	1.06	1.38	1.61
2	2.00	0.50	2.00	3.00	3.00
3	1.27	1.77	1.19	2.04	3.00
4	0.50	2.85	0.83	2.42	3.00
5	1.96	2.80	1.67	2.90	0.87
6	2.00	3.00	2.00	1.29	3.00
7	0.50	0.50	0.50	3.00	3.00
8	1.33	0.50	1.21	2.06	1.69
9	2.00	0.70	2.00	1.29	0.50
10	2.00	1.84	1.30	0.50	1.80
11	1.27	1.77	1.19	2.04	3.00
12	0.50	3.00	0.76	0.50	2.95
13	1.02	1.43	0.50	0.81	1.20
14	1.33	0.50	1.21	2.06	1.69
15	0.50	1.77	2.00	1.55	1.81
16	1.15	3.00	1.92	0.50	0.50
17	2.00	1.84	1.30	0.50	1.80
18	0.50	0.50	0.78	0.50	3.00
19	2.00	0.77	1.60	1.30	2.99
20	2.00	0.50	0.50	0.84	3.00
21	0.50	0.50	1.40	0.50	0.50
22	0.50	3.00	2.00	3.00	3.00
23	2.00	1.00	0.50	3.00	0.50
24	1.35	1.79	2.00	3.00	2.04
25	1.02	1.43	0.50	0.81	1.20
26	0.50	1.77	2.00	1.55	1.81
27	0.86	0.87	2.00	3.00	0.50
28	1.53	3.00	0.50	3.00	2.38
29	1.10	0.50	2.00	0.50	3.00
30	0.89	1.49	0.50	3.00	1.45
31	2.00	3.00	0.50	1.13	0.50
32* (PRS)	1.00	1.00	2.50	2.50	2.50

* PRS medium is used as a comparison in treatment #32

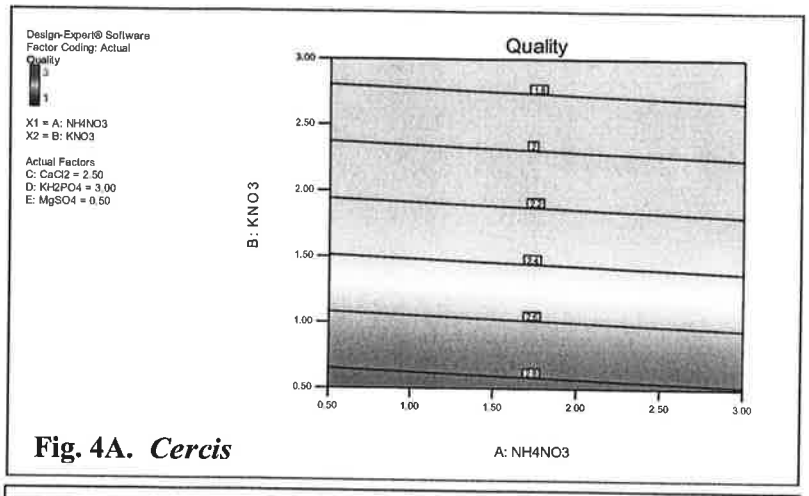

Fig. 4A. *Cercis*

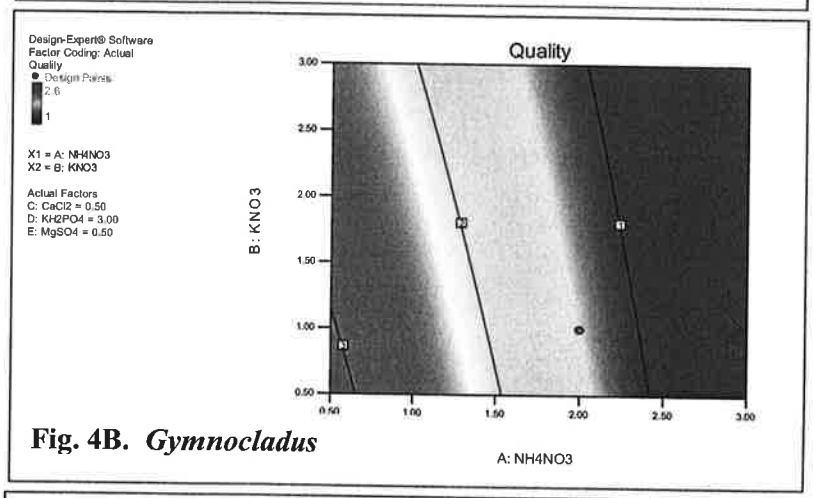

Fig. 4B. *Gymnocladus*

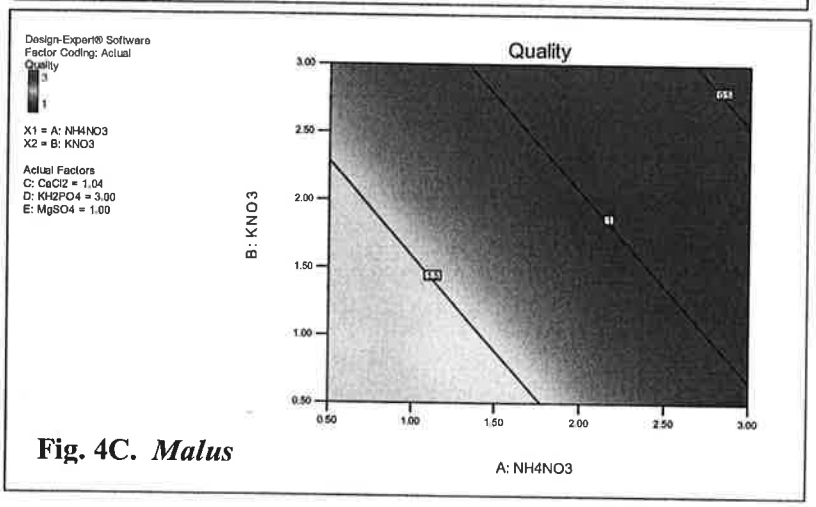

Fig. 4C. *Malus*

Fig. 4A *Cercis*, B *Gymnocladus*, and C *Malus*: Rating scale of 1=low quality (dark blue area) to 3=high quality (orange to red area) is indicated on the graphs. Design points are noted with a red dot if present in the design space represented by the graph.

Experiments in progress:

Carbon source can be a factor in shoot growth. In this study we compared two additional carbohydrates (glucose 30 g/L and sorbitol 35 g/L) with sucrose (30g/L). This experiment is currently ongoing. After repeated experiments the data will be analyzed sometime in early 2018. *Cercis canadensis* in Fig. 5 currently shows very different growth responses on the alternative carbohydrates.

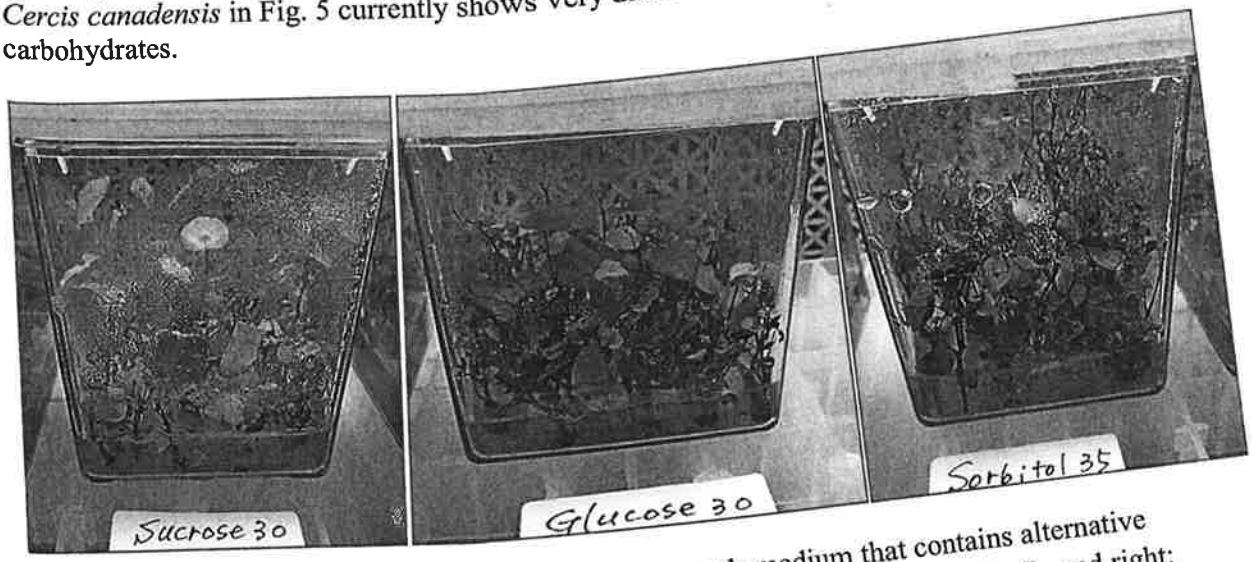

Fig. 5. *Cercis canadensis* currently cultured on growth medium that contains alternative carbohydrate sources (photos from left: sucrose 30 g/L, middle: glucose 30 g/L, and right: sorbitol 35 g/L).

This project is continued to 2018.