Midyear report to the Oregon Department of Agriculture (ODA)/Oregon Association of Nurserymen (OAN) November, 2014

Project Title: Improved Mineral Nutrition for Hazelnut Micropropagation

PIs: Sugae Wada, Ph.D. Department of Horticulture, Oregon State University, 4017 ALS Bldg. Corvallis OR 97331-7304, 541-971-0010, FAX 541-738-4205
Email: sugae.wada@oregonstate.edu
Barbara Reed, Ph.D.USDA-ARS National Clonal Germplasm Repository
33447 Peoria Road, Corvallis OR 97333-2521, 541-738-4216, FAX 541-738-4205,
Email: Barbara.Reed@ars.usda.gov

Cooperators: Ms. Melekşen Akin, OSU Department of Horticulture graduate student.

Background and Justification:

Hazelnuts are micropropagated commercially, but there are wide variations in growth response among cultivars from good growth to impossible to propagate. There is a need for a practical procedure to develop improved media formulations to suit these diverse cultivars. Media development has typically involved testing existing formulations to find one that provides adequate growth and development. We implemented studies using a response surface design and determined the main factors driving the growth of diverse hazelnut cultivars (Hand, 2013). The first part of the study was designed to determine what mineral nutrients were driving *C. avellana in vitro* shoot growth. Hazelnut genotypes 'Dorris,' 'Felix,' 'Jefferson', OSU 880.054, and 'Sacajawea' were used with 33 treatments for modeling. Multifactor response surface analysis projected that optimum shoot proliferation was greatly influenced by the NH_4NO_3 to $Ca(NO_3)_2$ ratios, mesos, and minors. These factors were important to overall quality and shoot length for all genotypes (Fig. 1). The graphs show some improvements for each genotype with changes in the various nutrient components, but there are still some deficiencies in shoot quality as seen in the photographs (Fig. 2). Minor nutrients had the biggest effect, and a follow-up study on minor nutrients determined the effects of the individual minor-mineral nutrients (including nickel) on hazelnut shoot growth with three cultivars, 'Dorris,' 'Jefferson,' and 'Sacajawea'. Six factors, H_3BO_3, $CuSO_4$, $MnSO_4$, Na_2MoO_4, $Zn(NO_3)_2$, and $NiSO_4$, at 0.5× to 4.0× DKW medium concentrations (Driver and Kuniyuki, 1984), were tested in a response surface design with 39 treatment combinations. Ni, not present in DKW, ranged from 0 to 6 µM. High concentrations of B, Mo, and Zn increased overall shoot quality, length and multiplication. There were many significant interactions. Improved growth and shoot quality in 'Dorris' and 'Jefferson' required increased amounts of B, Mo, and Zn with low Cu and Mn while 'Sacajawea' required increased B, Cu, Zn, and Ni (Fig. 2).

The diverse responses of these cultivars confirmed that nutrient uptake or utilization varied by genotype. In the initial study, improved shoot quality was also highly influenced by nitrogen components [NH_4NO_3 and $Ca(NO_3)_2$] and mesos ($MgSO_4$, KH_2PO_4) and K_2SO_4 for most of the cultivars tested. The next steps in developing improved media formulations require optimization of the mesos components, the ammonium and nitrate ratios and total N amounts**.** We are currently propagating Eastern Filbert Blight resistant selections produced by the OSU breeding program and they will be the focus of this study. This study will complete the testing for improved mineral nutrients of hazelnut.

Overall objective: Develop improved media for a wide range of hazelnut cultivars by altering the mineral nutrients. Specifically test to determine which ions have the most impact on growth. Develop optimized media and transfer that information to the commercial micropropagation industry. Test the final optimized growth medium on a wide range of cultivars.

Materials and methods:

Shoots of *Corylus avellana* hazelnut cultivars Dundee, Dorris, Jefferson, Wepster and Zeta, were used for this initial ion experiment. This study was designed to investigate the effects of different ion concentrations (Supplement 1) within the medium on the response of the five hazelnut cultivars using statistical software for response surface design analysis. There were 23 treatments and standard DKW medium salts and our current 'Hazelnut 2013 Medium' were used as controls (Supplement 2).

Data: Shoot quality is a subjective visual assessment of shoot vigor and form: 1=poor, 2=moderate and 3=good. Shoots longer than 5 mm will be counted. The longest shoots will be measured in millimeters. Leaf color will be rated 1= yellow, 2=light green, and 3=dark green. Callus size was rated: 1=callus > 2mm, 2=callus ≤ 2 mm, and 3=absent. Leaf size rated: 1=small, 2=medium, 3=large. Data was analyzed using Design Expert software.

Results

In this study the ion combinations lacking NH_4^+ did not support growth and resulted in dead shoots. However, some of the other combinations were excellent. The general response was that ammonium, magnesium, phosphorous and sulfate were best at high concentrations while the calcium requirement was low. Jefferson varied from the other genotypes in that the best growth was with low phosphate and low ionic strength (Table 1).

The design model was significant for shoot quality for all five genotypes ($P<0.001$). Ammonium and the total ionic amount were significant for four of the five while calcium and phosphorous were significant for three. Magnesium and sulfate were significant for two genotypes. There were many interactions. Quality graphs show the projected best regions of growth (Fig. 1). Ammonium was also an important factor in shoot multiplication (Data not shown).

Two general patterns emerged for ion utilization in the graphs (Fig. 1) and statistical analysis (Table 1). Four of the cultivars required high levels of all ions except calcium (low) and high ionic strength; 'Jefferson' required all ions high except PO_4 and low ionic strength. Treatment 15 fits the requirements for a general formula with low Ca and all other ions high. Treatment 1 fits the pattern for ‘Jefferson’ with low Ca and low ionic strength. The diverse growth responses of the five cultivars to various treatments is shown in Fig. 2. ‘Dorris’ and ‘Dundee’ grown on Treatment 15 have improved growth over the *Corylus* 2013 Medium, but the other three cultivars do not show any improvement on the treatments shown. Treatment 1 should be good for ‘Jefferson’, but it was not an improvement on the *Corylus* 2013 Medium (Fig. 2).

Table 1. Response of five hazelnut cultivars to ion concentrations and total ionic strength.

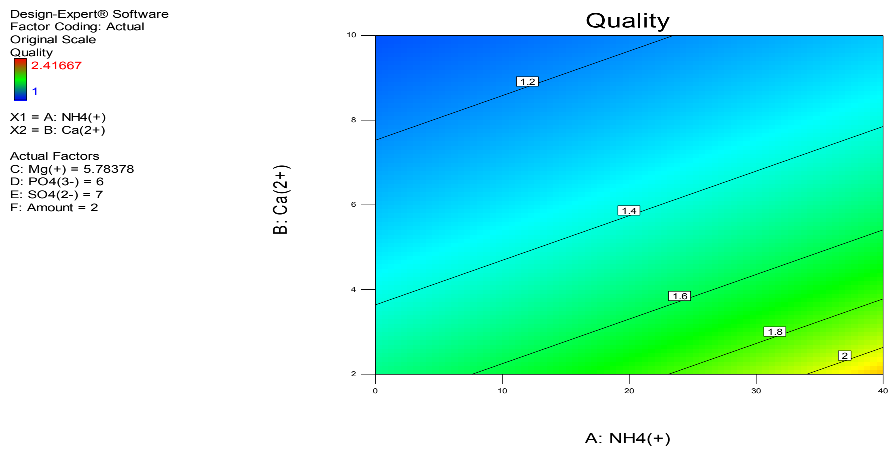
Cultivar	NH_4	Ca	Mg	PO_4	SO_4	amount ionic strength
Range tested	0 - 40	2 - 10	2 - 10	3 to 10	4 - 12	0.5 - 2x
Dorris	High	Low*	Med	Med	Med	2x*
Dundee	High*	Low*	M-H	High*	Med*	2x*
Jefferson	High*	High*	High	Low*	High*	0.5*
Wepster	High*	Low	High*	High*	High	2x
Zeta	High*	Low	High*	High	High	2x*
General formula	High	Low	High	High	High	High
Jefferson	High	High	High	Low	High	Low

*Significant factors ($P<0.05$)

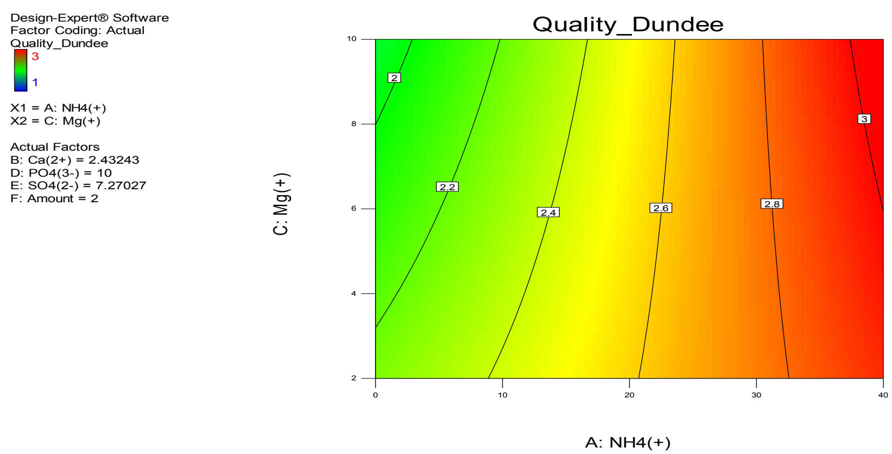
Conclusions

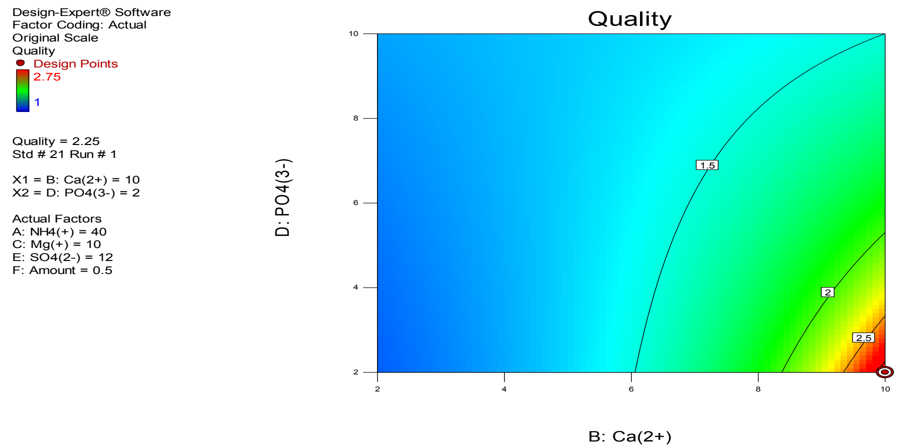
The hazelnuts in this study were all influenced by ammonium ions and required high levels of ammonium for the best growth. As was seen in earlier studies, hazelnuts are diverse in their backgrounds and their mineral nutrient requirements. This information and some follow up studies will be used to produce several medium formulations and those will be tested on a wide range of *Corylus* germplasm. The next study is now in progress.

Dorris



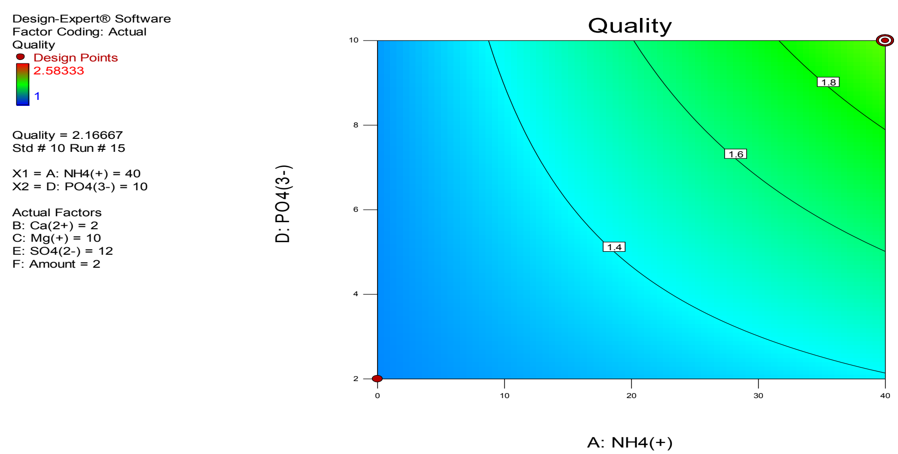
Dundee





Jefferson

Figure 1. Quality graphs of hazelnut cultivars indicating the amount of ionic strength and the concentrations of each of the ions tested. Red indicates the highest quality, blue the lowest quality.



Wepster

Zeta

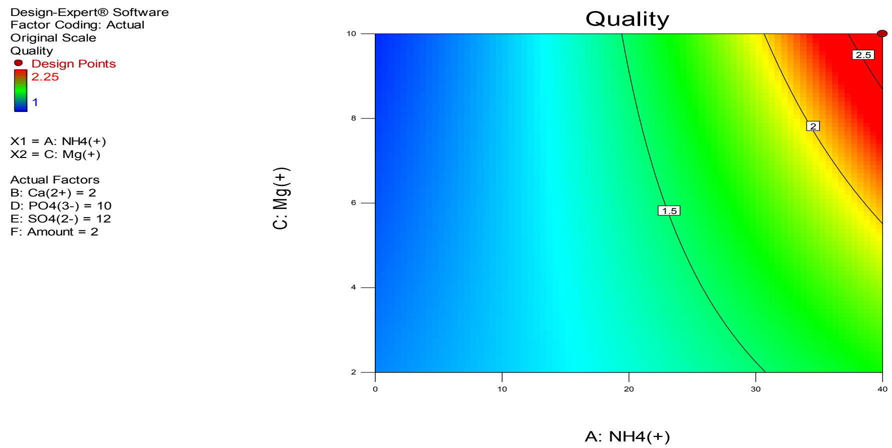


Figure 1. Quality graphs of the five hazelnut genotypes indicating the amount of ionic strength and the concentrations of each of the ions tested. Red indicates the highest quality, blue the lowest quality

Figure 2. Comparison of some of the better treatments (Supp. 1) for the cultivars.

Supplement 1. Ion formulations ($mg.L^{-1}$) of the treatments.

Treatments	NH_4^+	Ca^{2+}	Mg^{+2}	PO_4^{2-}	SO_4^{2-}	Amount % DKW
1	40	10	10	2	12	0.5
2	0	10	10	10	12	2
3	40	2	2	2	12	2
4	0	2	2	2	2	2
5	40	10	10	2	2	0.5
6	0	2	2	10	2	0.5
7	20	6	6	6	7	1.25
8	0	10	10	2	2	0.5
9	0	2	10	10	2	2
10	0	10	2	10	12	0.5
11	40	2	2	10	2	2
12	40	2	10	2	2	0.5
13	0	10	2	2	12	2
14	40	10	2	10	12	2
15	40	2	10	10	12	2
16	40	2	2	10	12	0.5
17	0	2	10	10	12	0.5
18	0	2	10	2	12	2
19	40	10	10	10	2	0.5
20	0	10	2	10	2	2
21	40	10	10	2	2	2
22	40	10	2	2	2	0.5
23	0	2	2	2	12	0.5
DKW 24	18	9.3	3	1.95	12	1

Supplement 2. Mineral salt composition (mg.L^{-1}) of the treatments used.

Treatment	KNO_3	KH_2PO_4	K_2SO_4	$MgSO_4$ * $7H_2O$	$Mg(NO_3)_2$ * $6H_2O$	$Ca(NO_3)_4$ * $4H_2O$	NH_4 NO_3	$(NH_4)_2SO_4$	$NH_4H_2PO_4$
1	506	0	0	0	641	590	280	396	58
2	4651	1361	349	2465	0	2362	0	0	0
3	5257	0	0	0	513	472	1121	1586	230
4	9099	272	0	493	0	472	0	0	0
5	506	0	0	0	641	590	680	66	58
6	2073	340	0	123	0	118	0	0	0
7	3539	0	0	0	962	886	0	578	431
8	1466	68	0	123	513	590	0	0	0
9	6673	1361	0	493	2051	472	0	0	0
10	1163	340	436	123	0	590	0	0	0
11	5257	0	0	0	513	472	2081	264	1150
12	910	0	0	0	641	118	680	66	58
13	5460	272	1743	493	0	2362	0	0	0
14	3640	0	0	0	513	2362	480	1586	1150
15	3640	0	0	0	2564	472	480	1586	1150
16	1314	0	0	0	128	118	120	396	288
17	1567	340	87	616	0	118	0	0	0
18	7077	272	349	2465	0	472	0	0	0
19	506	0	0	0	641	590	520	66	288
20	6673	1361	0	493	0	2362	0	0	0
21	2022	0	0	0	2564	2362	2721	264	230
22	910	0	0	0	128	590	680	66	58
23	1769	68	436	123	0	118	0	0	0
DKW 24	2803	0	85	370	0	1098	0	530	112