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Project Title: Improved Mineral Nutrition for Hazelnut Micropropagation

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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. 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 scompounds 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 Dorris, Wepster and Zeta, were used for this salt factors experiment. This study was designed to investigate the effects of four meso and two nitrogen compounds on the response of the three hazelnut cultivars using statistical software for response surface design analysis. There were 40 salt treatments that included altered micronutrients (Hand and Reed, 2013) and the standard DKW medium with was used as the control.

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

Quality: There were significant models for improved quality for all three genotypes, but the response to particular compounds varied. KH_2PO_4 and K_2SO_4 affected the quality of all of the genotypes ($p<0.05$). NH_4NO_3 was a significant factor for the quality of 'Wepster' and 'Zeta' ($p<0.01$). For 'Dorris', interactions of NH_4NO_3 *x* $Ca(NO_3)_2$ and $Ca(NO_3)_2$ *x* $MgSO_4$ all had an impact ($p<0.05$). All of the genotypes required very high KH_2PO_4 and low K_2SO_4 concentrations for best plant quality while the need for NH_4NO_3 was low. The salt requirements for best quality, as well as the treatments with better quality with comparison to the control, were similar for all the genotypes as illustrated by 'Wepster' (Fig. 1).

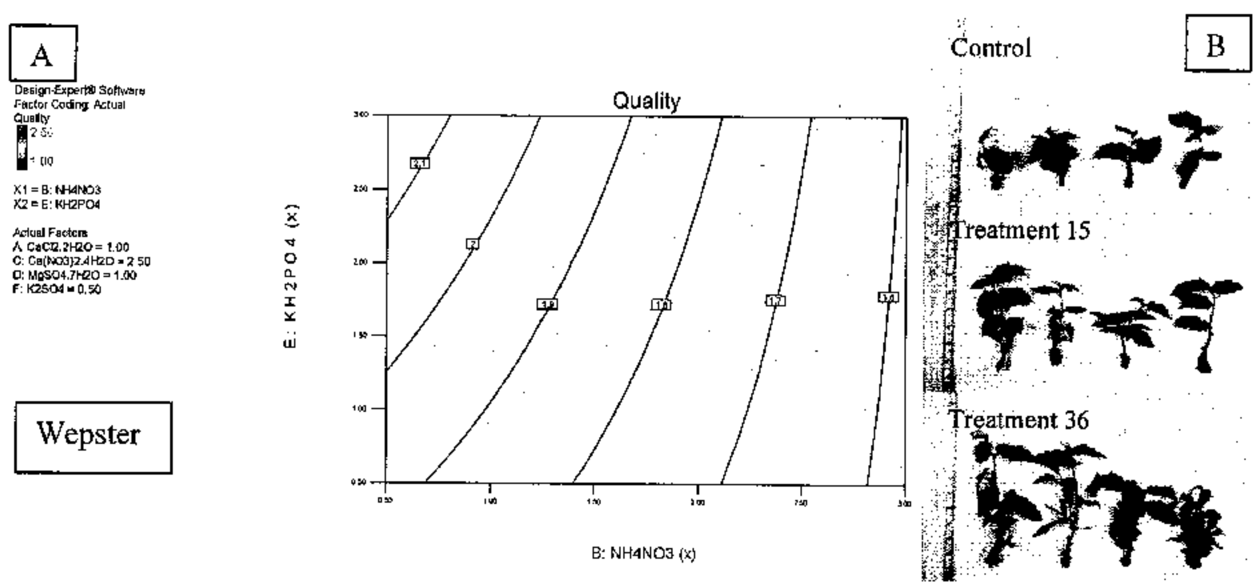

Fig. 1. The graph projecting the conditions required for the highest quality for 'Wepster' shoot cultures (A) and a comparison of shoots grown on the control and improved treatments (B). Similar results were seen for 'Dorris' and 'Zeta'.

Overall the most important quality factor was the concentration of NH_4NO_3. When the NH_4NO_3 was $\leq$ 1.7× DKW concentration, quality was better than at higher concentrations. In addition higher KH_2PO_4 and $Ca(NO_3)_2$concentrations (>2×) were required.

Shoot length: 'Dorris' required high KH_2PO_4 and $Ca(NO_3)_2$, but low NH_4NO_3 for ideal shoot length of 40 mm. 'Wepster' showed the same requirements for longer shoots (40 mm) as well, but $Ca(NO_3)_2$did not affect the response. 'Zeta' required only a low NH_4NO_3 concentration for good shoot length.

Shoot number: Low NH_4NO_3 was required for high shoot number for all three genotypes. There were interactions with other compounds as well. The lowest NH_4NO_3 and the highest $CaCl_2$ and K_2SO_4 improved ($p<0.05$) shoot number of 'Wepster'. 'Dorris' projected a high shoot multiplication rate (4.5) with the highest KH_2PO_4 and the lowest $MgSO_4$ and K_2SO_4 amounts ($p<0.05$). There was little improvement for 'Zeta'.

Leaf responses: Leaf color response were meaningful for 'Dorris' and 'Wepster' ($p<0.001$), but not for 'Zeta'. Increased nitrogen compounds improved leaf color for 'Dorris'. Shoots of 'Wepster' required the lowest K_2SO_4 and the highest NH_4NO_3 concentration for good leaf color. Both cultivars had additional interactions involving leaf color. Leaf size models were significant ($p<0.01$) only for 'Dorris'. Leaf size increased with high levels of $Ca(NO_3)_2$, NH_4NO_3, KH_2PO_4 and low amounts of K_2SO_4 and $CaCl_2$.

Callus: Responses of 'Wepster' and 'Zeta' were significant for callus formation ($p<0.05$) and the most effective factor was NH_4NO_3 ($p<0.001$). High concentrations of NH_4NO_3 (> 1.7×) and K_2SO_4 (> 2×) had the least callus (2.6) for 'Wepster'. 'Zeta' required high amounts of NH_4NO_3 (> 1.7) and K_2SO_4 (> 2×) and the lowest concentrations (0.5×) of KH_2PO_4 and $MgSO_4$for low amounts of callus (2.8).

Summary

The overall driving factor was lower NH_4NO_3 concentrations and higher $Ca(NO_3)_2$ and KH_2PO_4 (Table 1). In general half of the DKW NH_4NO_3 concentration and 2× the KH_2PO_4 with some increase in $Ca(NO_3)_2$ provided the best quality, shoot length and shoot number. Callus was decreased with increasing NH_4NO_3 so if excessive callus is a problem the NH_4NO_3 should be increased. Trials are in progress for several improved media and a range of genotypes.

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.

Table 1. Summary of results.

	Quality	Shoot length	Shoot number	Leaf color	Leaf size	Callus
Dorris	low NH_4NO_3 x high $Ca(NO_3)_2$ $Ca(NO_3)_2$ x $MgSO_4$ high KH_2PO_4	low NH_4NO_3 high $Ca(NO_3)_2$ high KH_2PO_4	low NH_4NO_3 high KH_2PO_4	low NH_4NO_3 high $Ca(NO_3)_2$	high $Ca(NO_3)_2$ high KH_2PO_4	high NH_4NO_3
Wepster	low NH_4NO_3 x high $Ca(NO_3)_2$ high KH_2PO_4	low NH_4NO_3 high KH_2PO_4	low NH_4NO_3 high $CaCl_2$ low K_2SO_4	low NH_4NO_3 high $MgSO_4$ low K_2SO_4		high NH_4NO_3
Zeta	low NH_4NO_3 x high $Ca(NO_3)_2$ high KH_2PO_4	low NH_4NO_3	low NH_4NO_3 low $Ca(NO_3)_2$			high NH_4NO_3

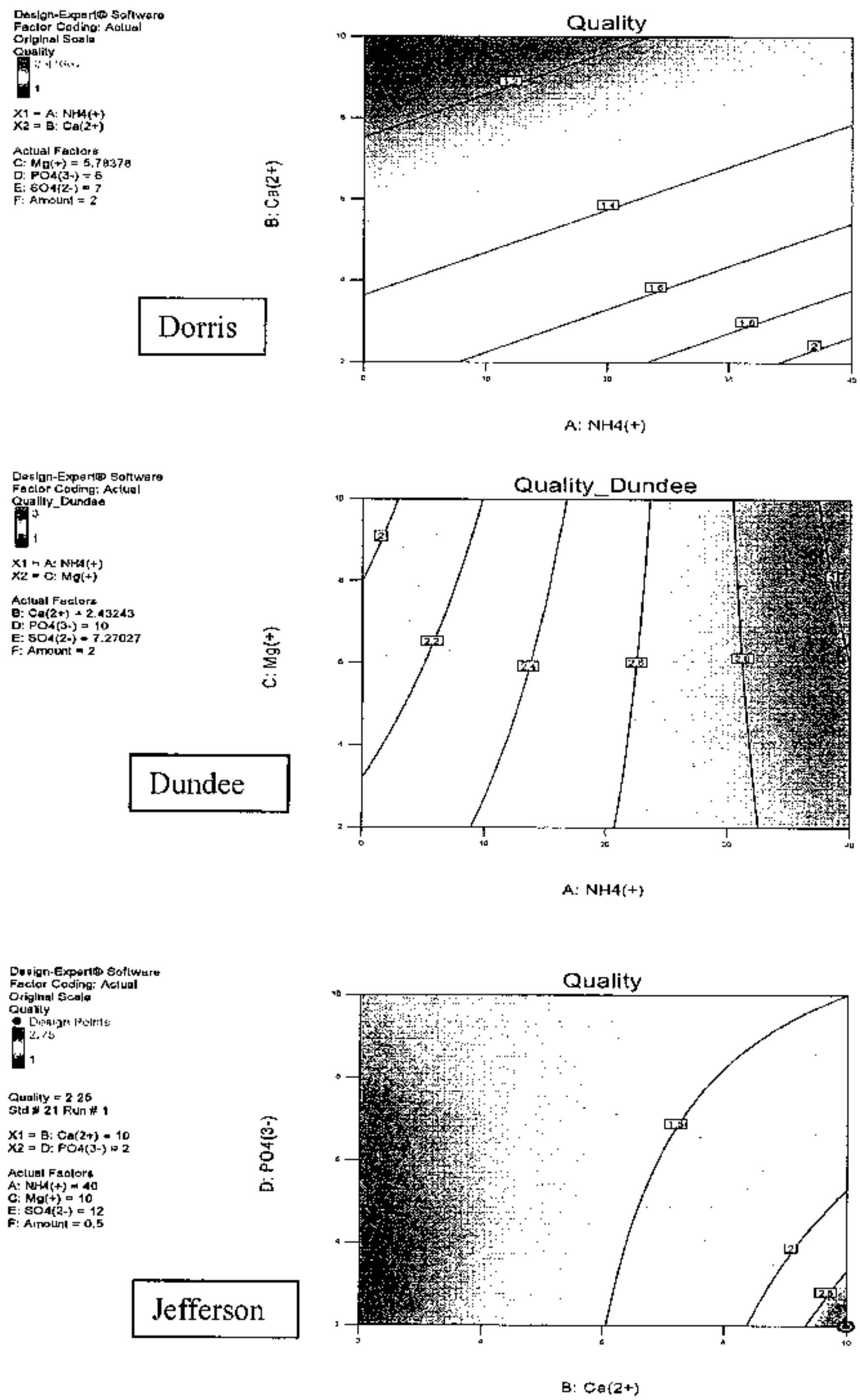

Figure 1. Quality graphs of hazelnut cultivars indicating the amount of ionic strength and the concentrations of each of the ions tested. Red indicates the highest quality, blue the lowest quality.

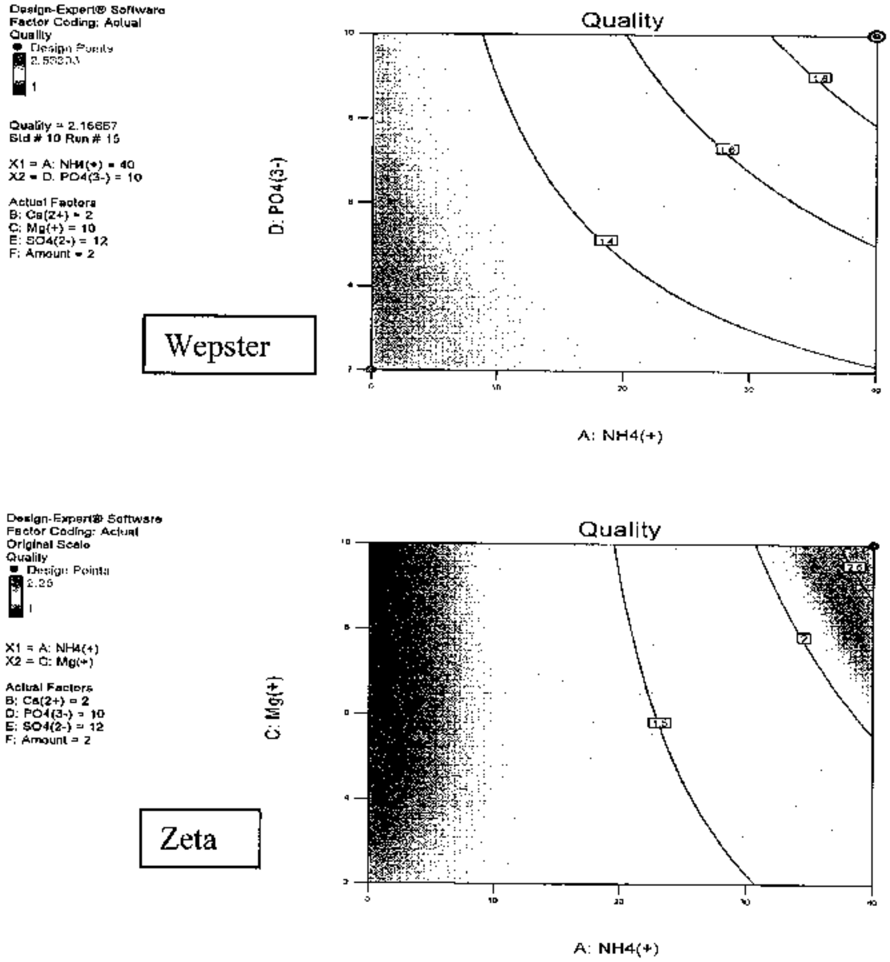

Figure 1. Quality graphs of the five hazelnut genotypes indicating the amount of ionic strength and the concentrations of each of the ions tested. Red indicates the highest quality, blue the lowest quality.

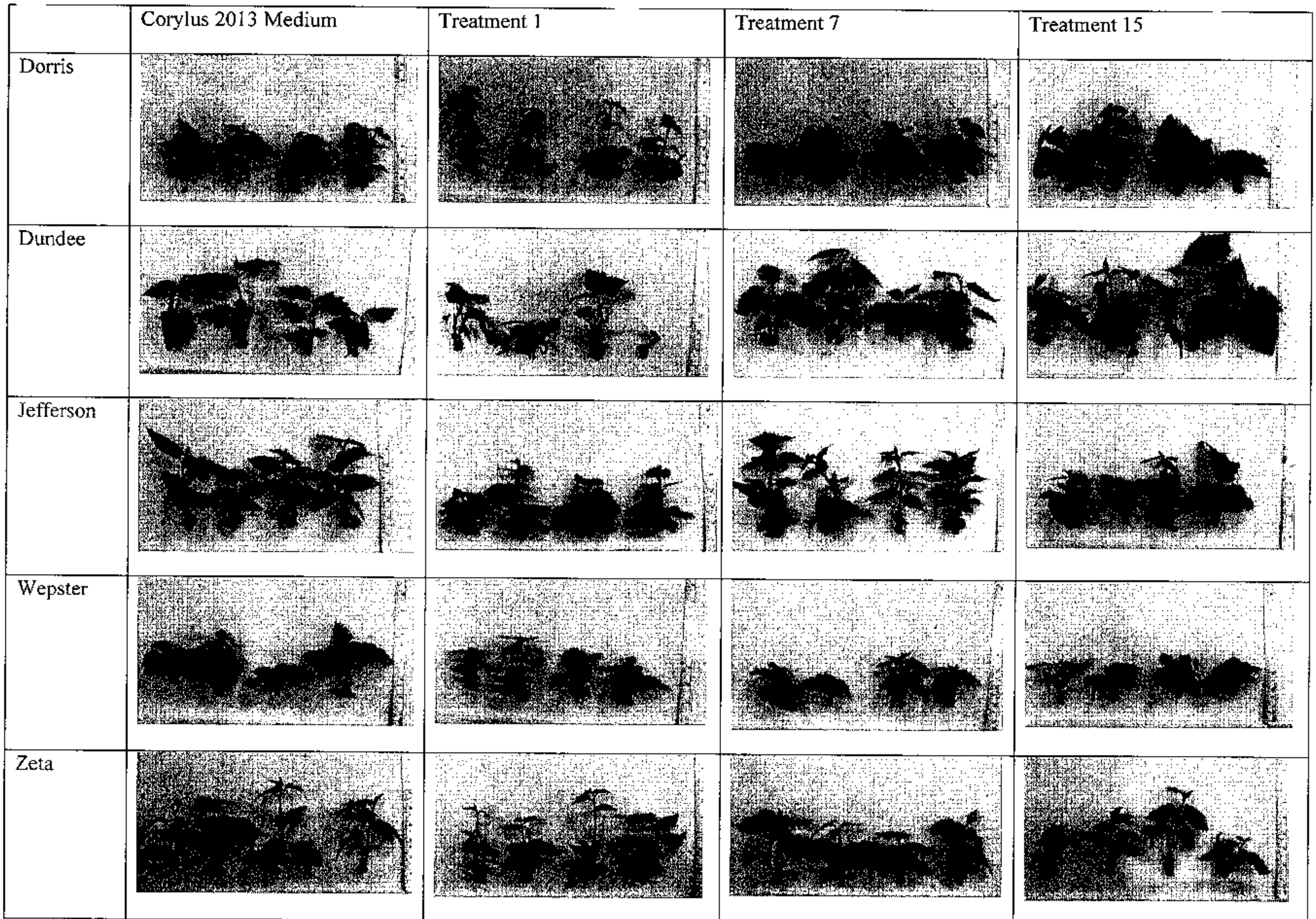

Figure 2. Comparison of some of the better treatments (Supp 1) for the cultivars.

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_4NO_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