

Optimization of media based on Design Expert optimization function

The Design Expert (DE) optimization function was used to predict the best combination of mineral nutrients for each genotype. Data from the responses were compared to set criteria of an ideal plant, and predicted combinations of the nutrient factors were produced. Response criteria for optimized hazelnut micropropagation were set at: quality rating of three, three shoots per plant, shoot length 30-60 mm, no callus, and medium leaf size (rating of two).

Materials and Methods

Based on optimization suggestions from DE software, the five cultivars were grown on four optimized treatments and a DKW control (Table 1). Growth conditions were the same as mentioned earlier except each treatment consisted of three boxes with 5 shoots (2.5 cm) for each genotype. Three plants taken from standard points in each box were examined for four responses (n=9). Quality rating, shoot length, shoot number and callus rating were recorded. ANOVA was completed using SAS software and DE.

Results and Discussion

The five trial treatments (Table 1) produced good growth for many of the genotypes but the results were generally inconclusive (Fig. 1, 2). Overall ANOVA indicated that only shoot length and callus were significant for genotypes at $p \leq 0.05$ and only shoot length by treatment (Table 2).

For individual genotypes there were significant differences in quality compared to the control (Treatment C) for 'Dorris' (Treatment B) and 'Felix' (Treatment A). Shoot length was significant for 'Felix' (Treatment A). Callus was significantly decreased for 'Dorris' with all treatments compared to the control and for OSU 880.054 on treatment E (Fig. 1, 2).

Table 1. Experimental nutrient combinations tested on hazelnuts. Levels were based on the projected “best” treatments from the design space. Treatment C (control) was DKW medium.

Treatment	NH ₄ NO ₃	Ca(NO ₃) ₂	MgSO ₄	KH ₂ PO ₄	K ₂ SO ₄	Minor nutrients
A	0.5	1.5	1.5	1.5	1	2
B	1	1.5	1.5	1.5	1	2
C control	1	1	1	1	1	1
D	1.2	0.5	2	2	1	2
E	1	1.7	1.5	1.5	1.3	2

Table 2. ANOVA for data from four experimental “optimized” media from Design Expert and the DKW control treatment (C).

P-values				
	Shoot Length		Quality	
Genotype	0.1594	0.0024	0.4547	0.0002
Treatment	0.1007	0.0311	0.8328	0.0516

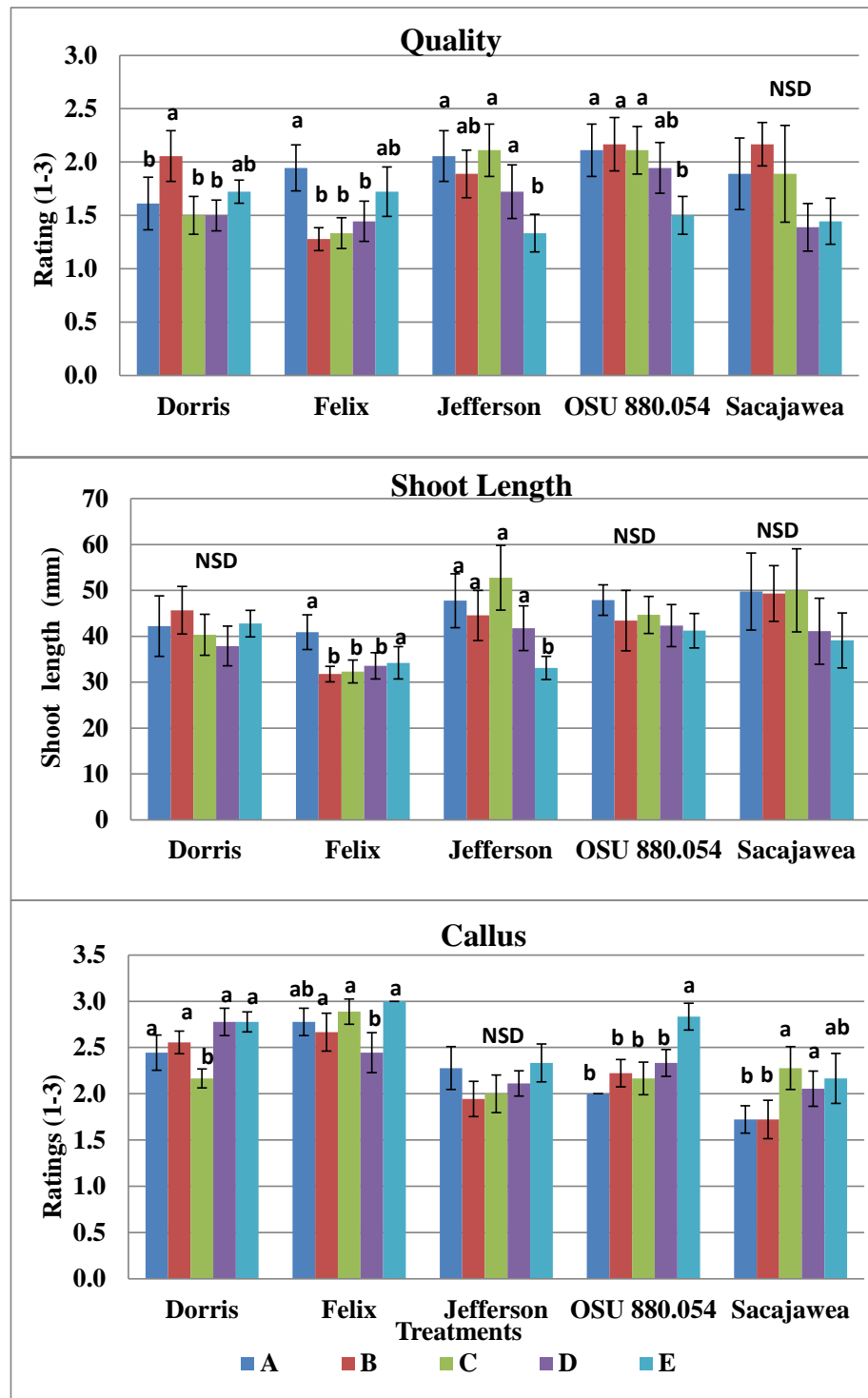


Fig. 1. Growth responses of ‘Dorris,’ ‘Felix,’ ‘Jefferson,’ OSU 880.054, and ‘Sacajawea’ to five test medium formulations (A, B, C, D, E). NSD = no significant difference at $p \leq 0.05$.

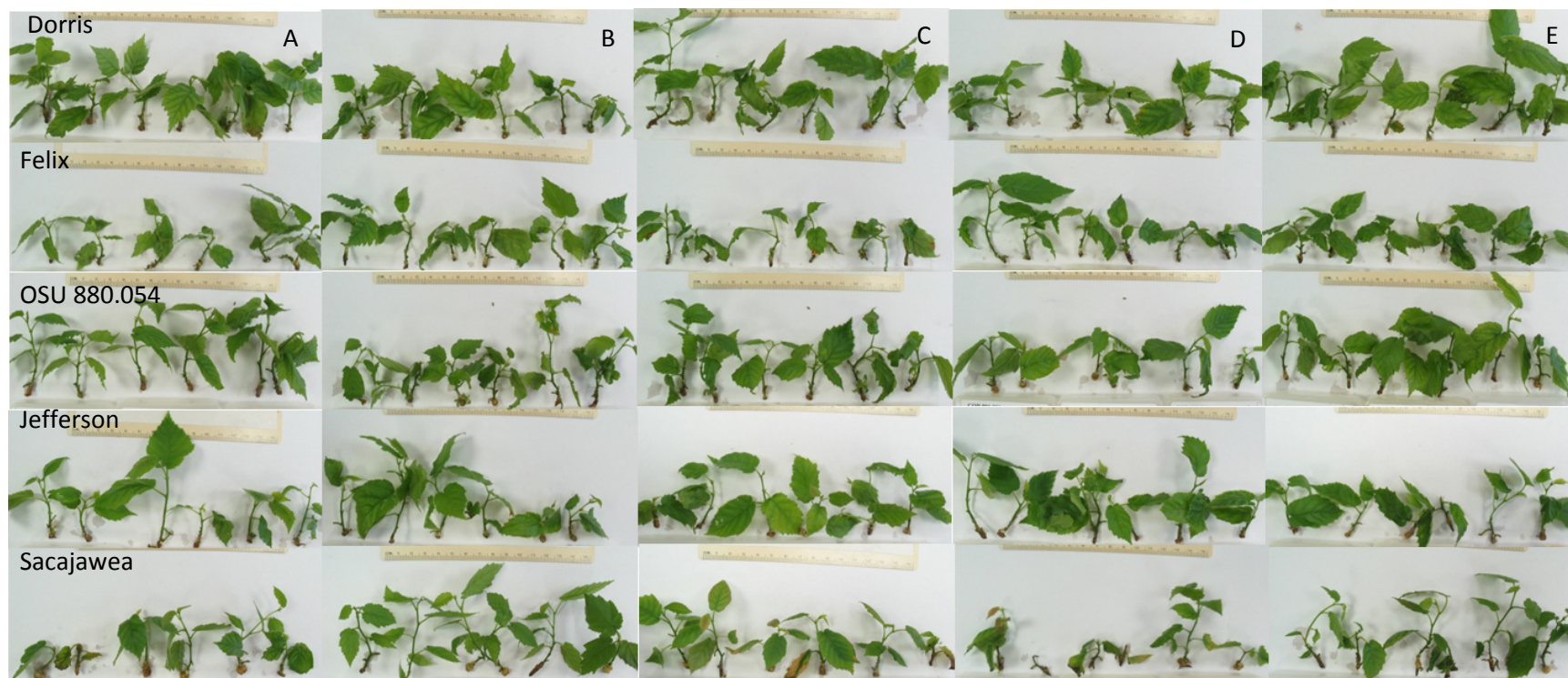


Fig. 2. Hazelnut cultivars grown on four experimental media (A,B,C,E) suggested by the Design Expert program and the control DKW medium. A. $0.5\times \text{NH}_4\text{NO}_3$, $1.5\times \text{Ca}(\text{NO}_3)_2$, $1.5\times$ mesos, $1\times \text{K}_2\text{SO}_4$, $2\times$ minor nutrients. B. $1\times \text{NH}_4\text{NO}_3$, $1.5\times \text{Ca}(\text{NO}_3)_2$, $1.5\times$ mesos, $1\times \text{K}_2\text{SO}_4$, $2\times$ minor nutrients. C. DKW mineral nutrients at $1\times$. D. $1.2\times \text{NH}_4\text{NO}_3$, $0.5\times \text{Ca}(\text{NO}_3)_2$, $2\times$ mesos, $1\times \text{K}_2\text{SO}_4$, $2\times$ minor nutrients. E. $1\times \text{NH}_4\text{NO}_3$, $1.7\times \text{Ca}(\text{NO}_3)_2$, $1.5\times$ mesos, $1.3\times \text{K}_2\text{SO}_4$, $2\times$ minor nutrients.