AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Improved Mineral Nutrition for Blueberry Micropropagation

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SUMMARY: Blueberry cultivars of widely-divergent genetic backgrounds are often difficult to culture on one standard growth medium. This study used the mineral components of Woody Plant Medium (WPM) as factors to optimize growth medium for five diverse genotypes: *Vaccinium arboretum* Marshal (Sparkleberry), *V. ashei* Reade 'Ochlockonee' (Rabbiteye), *V. corymbosum* L. 'Draper' (Northern High Bush), *V. corymbosum × ashei* hybrid 'Misty' (Southern High Bush), and *V. corymbosum × V angustifolium* Aiton. hybrid 'Tophat' (Half High). Shoot cultures grown on standard WPM were compared to 40 treatments based on the mineral components of WPM. Factors were NH_4NO_3 and $Ca(NO_3)_2$ at 0.5 to 2× and K_2SO_4, and mesos (KH_2PO_4, $CaCl_2$ and $MgSO_4$) from 0.5 to 3×, and the combined minor nutrients from 0.5 to 4×. A response surface design was used for modeling the responses. Shoots grown on each treatment for 15 weeks were evaluated for shoot quality, multiplication, shoot length, stem width and color, leaf size, color and necrosis, shoot tip necrosis, callus production and hyperhydricity. The subjective quality rating was generally inclusive of the other factors. Mineral factors that influenced quality varied for the five genotypes. All five genotypes were greatly influenced by calcium nitrate and mesos but the impact of the other factors varied. Minor nutrients were best at the standard WPM concentration or at half strength. Each of the characteristics evaluated could be manipulated by varying the mineral nutrients. Final media will be formulated and tested.

OBJECTIVES:

1. **Overall objective:** Develop improved growth media for a wide range of blueberry cultivars and species by altering the mineral nutrients.
2. Determine changes in the mineral content (N, P, K, Mg, Ca, S, B, Mn, Zn, Cu, Fe) of the *in vitro* plants related to mineral nutrition and plant growth response.
3. Develop one or more optimized blueberry media and transfer that information to the commercial blueberry industry.

PROCEDURES: *Plant material*. Testing was performed on five blueberry genotypes: *Vaccinium arboretum* Marshal (Sparkleberry), *V. ashei* Reade 'Ochlockonee' (Rabbiteye), *V. corymbosum* L. 'Draper' (Northern High Bush), *V. corymbosum × ashei* hybrid 'Misty' (Southern High Bush), and *V. corymbosum × V angustifolium* Aiton. hybrid 'Tophat' (Half High).

Propagation. The medium used was woody plant medium (WPM) (Lloyd and McCown, 1980) salts, vitamins, iron and per L: 2 mg zeatin (PhytoTechnology Labs, Shawnee Mission, KS), 30 g sucrose and 7 g agar (PhytoTechnology Labs,) at pH 5.2. Shoot cultures were grown in Magenta MK-5 boxes with 65 ml

medium/box with a transfer to fresh medium every 5 weeks. Cultures were grown at 24°C under a 16-hour photoperiod with an average of 76 $\mu Mm^{-2}s^{-1}$ radiation provided by cool white fluorescent lamps.

Experimental design. An experimental design space was developed to model all possible combinations of five factors at five concentrations using Design-Expert software (Design-Expert, 2010). Five mineral nutrient factors for treatments were based on the salts of WPM: (1) NH_4NO_3, (2) $Ca(NO_3)_2$, (3) $CaCl_2$ and $MgSO_4$), (4) KH_2PO_4 and K_2SO_4, (5) and minor elements (B, Cu, Co, Mn, Mo and Zn) (Table 1). Experimental treatments (design points) were determined using the five factors at 5 levels to sample the design space consisting of all possible treatments. NH_4NO_3 and $Ca(NO_3)_2$ 0.5 to 2× .

Data. Eleven shoot responses were measured for six plants (or 12 shoots if internally replicated) for each treatment: quality (a subjective assessment of plant appearance (1 = poor, 2 = acceptable and 3 = good); shoot multiplication (shoots counted); shoot length (shoot clump measured); stem color (1=red, 2=pale green, 3=dark green), stem width (1=thin, 2=medium, 3=wide), leaf color (1 = red/yellow 2 = light-green, 3 = dark green); leaf size (1 = large, 2 = medium, 3 = small); leaf necrosis (1=complete, 2=partial, 3= absent) callus (1 large, 2 medium, 3 absent), shoot tip necrosis, hyperhydricity (1 much, 2 some, 3 absent).

Statistical analysis. The design space was modeled from the plant response at each design point using the mean of six (or 12) shoots. Some points were replicated with two duplicate Magenta boxes. For each measured response, the highest order polynomial model was analyzed by ANOVA where additional model terms were significant at the 0.05 level (Evens and Niedz, 2008; Niedz and Evens, 2006; Niedz and Evens, 2007). The software application Design-Expert® 8 (Design-Expert, 2010) was used for experimental design construction, model evaluation, and analyses.

SIGNIFICANT ACCOMPLISHMENTS: The first experiment determined that the nitrogen and mesos factors were very important for all five genotypes tested and some other nutrients varied among the genotypes. The second experiment studied the nitrogen levels and found mostly $Ca(NO_3)_2$ effects on shoot multiplication. A final mesos and micronutrient experiment is in progress. After this experiment new media will be formulated.

BENEFITS & IMPACT: Providing improved growth media for blueberries will allow for increased production of nursery plants and better, faster distribution of new cultivars. Developing media for diverse types of blueberries will improve the prospects for breeding cultivars with more diverse genetic backgrounds.

ADDITIONAL FUNDING RECEIVED: In-kind funding was provided by Fall Creek Nursery for all medium components, preparation, labor for planting and plant materials. ARS provided salary for Dr. Reed.

FUTURE FUNDING: This project should complete the needed work on blueberry and the growth media formulations developed can be distributed to micropropagation nurseries.