Validating four model medium formulations from mineral nutrition screenings and two commercially standard culture media (WPM/PBM) on diverse *Vaccinium* cultivars - 2021

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Project background and justification

Micropropagation is important for a wide range of nursery crops. However, because individual cultivars differ in their cultural requirements, developing growth media for specific and unique cultivars is often difficult. Micropropagation can be also difficult for some elite clonal stocks and is not well developed for newer cultivars or for some species that are used for advanced breeding. Blueberry production in Oregon was 134 million pounds in 2018, making Oregon one of the top blueberry producers in the USA. Many of the 14,500 acres of blueberry plants were produced through micropropagation at commercial nurseries in Oregon.

New tissue culture growth media are typically developed by minor modifications to "standard" media formulations. This approach, though useful for species or cultivars that respond well to the "standard" formulations, is not useful for the more difficult cultivars and species where growth is suboptimal. What is required is a systematic approach to efficiently evaluate the large number of components in a typical formulation (there are thirteen essential plant mineral nutrients). Design of Experiment software can be used to improve many aspects of micropropagation media (Niedz and Evens, 2007). These types of studies allow observation of unique responses related to the genetics of the plants studied. This approach is now used to develop optimized tissue culture media for a wide range of plants. Early studies developed medium to grow pears that had long been difficult to culture in vitro (Reed et al., 2013, Wada et al.,2013, Wada et al. 2014). A similar process produced an optimized growth medium for hazelnuts that allowed widespread use of in vitro plants for expanding the Oregon hazelnut industry (Hand et al., 2014; Akin et al., 2018).

Our initial studies of blueberry media using computer aided design and analysis provided models for five blueberry cultivars that may be useful for wider use in blueberry micropropagation. That study evaluated the effects of five groups of mineral nutrients on five cultivars and provided models for optimized growth medium formulations. Therefore, validating the four optimized model formulations from our prior studies by comparing them with some commercially most used blueberry culture media such as Woody Plant Medium (WPM) and Preece's Blueberry Medium (PBM), would allow determining their suitability and productivity, as well as wider use of the model formulations for cultivars with diverse genetic backgrounds.

Project objectives

1. Test a wide range of commercially important blueberry genotypes on WPM, PBM and four experimental formulations from the models developed in earlier studies.
2. Publish finalized blueberry formulations for used by the industry.

Methods and timelines

Plant materials: Similar genetically diverse blueberry cultivars will be used for study. Prior mineral nutrition screening studies were performed on five blueberry genotypes including hybrids (Table 1).

Shoot cultures will be grown in Magenta G-7 containers with 50 ml medium/box with a transfer to fresh medium every 5 weeks. Growth room conditions will be 24°C under a 16-hour photoperiod with an average of 76 $\mu Mm^{-2}s^{-1}$ radiation provided by cool white fluorescent lamps. Shoots will be planted on each treatment medium (Table 1) in triplicate boxes with 25 shoots per box. Cultures will be grown for 5 weeks and transferred twice to the same treatment medium for a total of 15 weeks of culture. Boxes for each cultivar will be randomized on the growth room shelf. Data will be taken at 15 weeks on 10 shoots in each box (at pre-designated locations in the box) and representative shoots will be photographed.

Table 1. Plant materials (five genotypes) used for initial screening of mineral nutrition.

Species	Cultivar	Note
Vaccinium arboretum Marshal	Sparkleberry	
V. ashei Reade	Ochlockonee	Rabbiteye
V. corymbosum L.	Draper	Northern High Bush
V. corymbosum × *V angustifolium* Aiton. hybrid	Tophat	Half High
V. corymbosum × *ashei* hybrid	Misty	Southern High Bush

Table 2. Test media –As a factor of the Woody Plant Medium concentration (x)

Experimental Formulations	Ca Nitrate	Ammonium Nitrate	Potassium Sulfate	Mesos (KH_2PO_4, $CaCl_2$ $MgSO_4$)
A*	1	1	3	2
B	2	1	3	3
C	2	2	1	1
D	2	2	1	3
WPM **	1	1	1	1
PBM ***	1	1	1	1

* Four formulations (A to D) chosen from our prior mineral nutrition screenings on five diverse blueberry cultivars (Table 1).

** Wordy Plant Medium (WPM) / *** Preece's Blueberry Medium (PBM)

Data. Eleven shoot responses will be measured for 10 plants per box for each treatment (n=30): 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. Data will be analyzed with analysis of variance (ANOVA) and means separation using SAS (SAS 2008).

Timeline:

Winter-Spring 2021: Multiply stock material for experiments.
Spring –Summer 2021: Run experiment first set three times for 4 weeks each.
Take data at 16 weeks.
Summer-Fall 2021: Run second set of experiment 3 times for 4 week intervals on the test medium.
Take data at 16 weeks.
Fall 2020: Data analysis and final report.

Budget summary:

The request is for a partial FTE of Assistant Professor (Sr. Res.) salary (Dr. Wada), student lab assistant and experimental supplies including lab chemicals.

Item	Year 2020
Salary (0. 15 FTE)	$13,600
Benefits (0. 55 %)	$7,480
Student worker (4h/ week)	$3,200
Supplies & chemicals	$1,200
Total	$25,480

Complementary budget items: Dr. Reed is retired and is volunteering her time. All software, overhead costs, a completely equipped tissue culture laboratory and growth room are included.

Benefit to the nursery industry

This study will validate the use of models for determining the best nutrition for in vitro blueberry plants. This will also test and validate two most used growth media for blueberry micropropagation in commercial nurseries. It will provide data on the variability of response of cultivars with diverse genetic backgrounds. It may also provide new growth media for 'difficult to propagate' blueberry cultivars.

The beneficiaries are micropropagation nurseries, commercial nurseries and growers. Improved growth media will allow faster propagation and make microplant production more profitable and higher productivity so that the plant materials more available to the nursery industry.