Micropropagation of apple rootstock Geneva 214 - 2020

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Project background and justification

Woody plants are often difficult to propagate by either traditional or in vitro techniques. Many fruit trees are produced through micropropagation or micropropagation is the only means for multiplication, but there are wide variations in growth response among cultivars from good growth to impossible to propagate. In addition, newly developed apple rootstocks are difficult to propagate. Micropropagation provides rapid propagation of new cultivars and availability at any season. Geneva apple rootstocks are important to the nursery industry and they are difficult to propagate. The Geneva 214 rootstock, a highly demanded cultivar, will be the focus in this study.

Many genotype-specific nutrient media were developed empirically, however, these media are not effective for all cultivars. It is often difficult to optimize the mineral nutrition in the medium used for the growth of *in vitro* plants due to the complex chemical interactions of essential nutrients. Computer-aided design software is now available that can be used to plan complex experiments for improving the quality of in vitro cultures (Niedz and Evens 2007). The response surface methodology (RSM) approach uses computer design to provide a geometric design where all possible combinations of factors are sampled to provide an experimental design that both samples the design space and graphically models the remaining points. These models, based on plant growth data, provide information on which of the medium components are the most influential for the desired type of plant growth. The information obtained can be used to alter individual components of the medium to provide an optimal result. The individual mineral nutrient factors of a medium may also be identified to determine the response of the plant on each variation of mineral composition or to study mineral uptake and utilization.

Our laboratory has worked to improve micropropagation of both woody and herbaceous plants for over 30 years. We implemented studies using a response-surface design of experiments and determined the main nutrient factors driving the growth of diverse woody plants including *Pyrus* and *Corylus* species and cultivars and developed improved mineral nutrient growth media that greatly improved the growth of a large number of species and cultivars. These studies made it possible to micropropagate species and cultivars that were previously recalcitrant to in vitro culture and to greatly improve the growth of those that initially had marginal responses.

Our initial studies determined the correct growth regulators and carbon sources for micropropagation of Geneva 214. Now that the Geneva 214 rootstocks will grow moderately well, it is possible to optimize the mineral nutrients and develop a final optimized medium. The current study will optimize mineral nutrients using computer aided design and modeling and finalize a growth medium for Geneva 214.

Project objectives

1. Determine optimal mineral nutrition for Geneva 214 rootstock.
2. Test the finalized medium based on the model.
3. Develop final optimized growth medium for use by industry.

Methods and timelines

Culture condition: The base MS medium with changes developed in earlier studies will be used for stock plants. Medium was autoclaved for 20 min at 121 ºC and 15 psi. Chemicals were sourced from PhytoTechnology Laboratories (Shawnee Mission, KS). Shoots were grown in Magenta GA7 boxes (Magenta Corp. Chicago, USA) with 40 ml of medium in each box with a transfer to fresh medium every 4 weeks. Cultures were grown at 24°C under a 16-hour photoperiod with an average of 60 $\mu molm^{-2}s^{-1}$ radiation provided by cool-white fluorescent lamps.

Experimental design: The in vitro growth response experiment with MS medium nutrients will be developed with the software program Design-Expert® 8 (Design-Expert 2010). An experimental design was developed using Design Expert software (Stat-Ease, Minneapolis, MN) (Design-Expert 2010). Six mineral nutrient factors were used to develop design points (treatments) based on the MS salts: (1) NH_4NO_3, (2) KNO_3, (3) $CaCl_2$, (4) KH_2PO_4, (5) $MgSO_4$ and (6) minor elements (B, Cu, Co, Mn, Mo and Zn). Other medium components will be the same as used for the stock cultures. Experimental treatments (design points) were determined with Design Expert software using the six factors at five levels to sample the design space of all possible treatments. The composition of all mineral factors varied from 0.5 to 3× the MS concentration for a total of 40 treatments (Table 1). The experiment will require 420 shoots and will be done in two blocks.

Data and statistics: 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 size will be rated as 1=small, 2=medium, 3=large and leaf color will be rated 1= yellow, 2=light green, and 3=dark green. Callus size rated: 1=callus > 2mm, 2=callus ≤ 2 mm, and 3=absent. Any abnormal growth will be noted. The mean plant responses from the nine plants of each genotype grown in the same treatment for 12 weeks (three passages) will be used for analysis. The model will be generated from the plant response at each design point using the mean of six (or 12 if replicated) shoots. For each measured response, the highest order polynomial model will be analyzed by ANOVA where additional model terms are considered significant at the 0.05 level. The software application Design-Expert® 8 (Design-Expert 2010) was used for experimental design construction, model evaluation, and analyses.

Timeline:

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

Budget summary:

The request is for 0.17 FTE of Assistant Professor (Sr. Res.) salary and supplies (Dr. S. Wada).

Item	Year 2020
Salary (0. 17 FTE)	$13,600
Benefits (0. 55)	$7,480
Student worker (4h/ week)	$4000
Supplies & Equipment	$200
Total	$25,280

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

Benefit to the nursery industry

The beneficiaries are micropropagation nurseries, commercial nurseries and growers. Improved growth media for this apple rootstock will allow faster propagation and make microplant production more profitable and the plants more available to the nursery industry. Eventually, completing the medium optimization process will produce a new medium for improved propagation and enhanced productivity for micropropagation laboratories involved with producing rootstocks for nurseries.