Completing micropropagation of apple rootstock 'Geneva 214' - 2021

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Project background and justification

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. Completion of an improved growth medium for 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 prior 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 use the result of optimize mineral nutrients using computer aided design and modeling and finalize a growth medium from Covid-extended 2020 project. Then, continued in 2021 to combining these results with the growth regulator and carbon source data and testing formulations for growth response. These studies will conclude by determining the best combinations as a culture medium for Geneva 214.

Project objectives

1. Developing the final culture medium formulation for propagating Geneva 214 rootstock.
2. Test the finalized medium formulated with all combined factors (carbon sources, plant growth hormone, and active charcoal) from prior testing results.
3. Develop final optimized growth medium for use by industry and prepare publications.

Methods and timelines

Culture: 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. The experiment will require 420 shoots and will be done in two blocks. This completes the 2020 study.

Growth regulators, carbon source, activated charcoal effects: A second in vitro growth response experiment with Design-Expert will test growth regulators, carbon source and charcoal for the optimum effects on the best mineral nutrient medium. A three-factor design will be used with about 21 treatments in one block.

Final medium testing: The results of the first two studies will provide data for developing the final protocol and medium. Triplicate trials of test media will be conducted with the shoots grown on each test medium for three passages.

Data and statistics: For the Design Expert experiments, 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. SAS analysis (ANOVA, Means separation) will also be utilized as if needed.

The replicated trials of finalized medium components will be done with three boxes of each treatment and 9-16 shoots per box (depending on stock available). Each set will be passed to new medium at 4 week intervals so that the plants have 12 weeks of exposure to the treatment. Data

will be taken at 12 weeks on a range of growth responses as noted for the earlier experiments and analyzed with SAS statistical software.

Timeline:

Winter-Spring 2021: Run the three-factor study of growth regulators, carbon sources and activated charcoal on the best formulation of mineral nutrition study 2020 results.
Summer-Fall 2021: Run the optimized formulations from the final test in replicated trials.
Fall 2021: Data analysis, Test the final medium and prepare final report.

Budget summary:

The request is for a partial FTE of Assistant Professor (Senior Research) salary (Dr. Wada), 4 hour/week TC lab student assistant and experimental supplies.

Item	Year 2020
Salary (0. 15 FTE)	$13,600
Benefits (0. 55 %)	$7,480
Student worker (4h/ week)	$3,200
Supplies	$1,000
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 and in vitro 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. this study is completing the medium optimization process and will produce a new complete medium for improved propagation and enhanced productivity for micropropagation laboratories involved with producing demanded but difficult-to-propagate this apple rootstocks for nurseries.