FINAL PROJECT REPORT

Project Title: Optimization of rosaceae rootstock micropropagation

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Total Project Request: Year 1: $13,000

Other funding Sources - None

Total Project Funding: $13,000
Budget History

Budget History

Item	2008	2009	
Salaries	10,000		
Supplies	3,000		
Travel			
Miscellaneous			
Total	**13,000**		

NOTE: THIS IS A TWO-YEAR PROJECT WITH FUNDING REQUESTED ONLY FOR THE FIRST YEAR. SOME OF THE SUB-OBJECTIVES WILL BE ACCOMPLISHED IN THE COMING MONTHS.

OBJECTIVES

In order to rapidly multiply G 41 and G 935 rootstock material several parameters were tested and optimized. We had three main objectives to accomplish our goal of accelerating rootstock multiplication.

1. Media Optimization: This objective focuses on identifying optimal conditions of the growth media from the stage of multiplication to transfer of the rooted plantlets to soil in a green house. It has three sub-objectives:

A. Identify optimal growth media formulation for accelerated G 41 multiplication
B. Optimize multiple root formation
C. Standardize transfer of rooted plants to soil in a green house condition

2. Photobiological Regulation of Rootstock Multiplication: Light has the capacity to regulate plant growth.
A. Under this objective, we aim to optimize G 41 multiplication and/or multiple root formation using different light regimes

3. Temporary Immersion System (TIS): This is a novel micropropagation technology that simulates plant growth in nature, only in an abbreviated timeline. The objective is to compare plant growth rates in TIS vs traditional media.

SIGNIFICANT FINDINGS

1. None of the reported media or the recipes provided by commercial lab was found to be suitable for G 41 rootstock micropropagation.

2. It takes over 10 weeks for two-fold multiplication of a given G 41 explant.

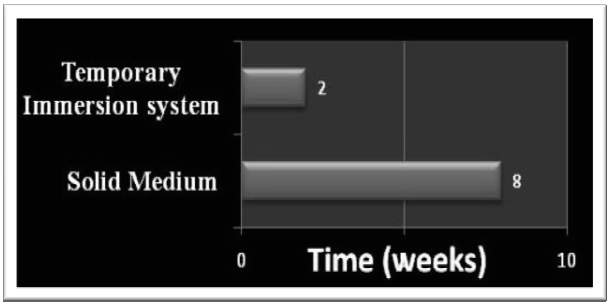
3. With the temporary immersion system the multiplication time can be reduced to two weeks.

RESULTS AND DISCUSSION

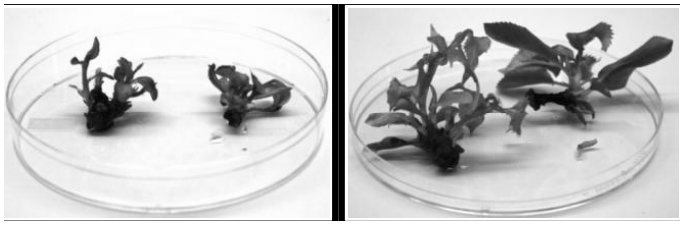
1. Media Optimization: The media commonly used in the commercial labs was based on the formulation described by Murashige and Skoog (1964). This media called MS Media was formulated for tobacco. We were quickly able to optimize media for healthy growth of G-41 by changing the nitrogen source in the media (Figure 1). Box on the right represents the media formulation provided by commercial labs. Healthy plant growth is routinely obtained on the media optimized in the laboratory. Currently we are standardizing rooting. Our goal is to obtain multiple roots that enable better survival of the explant in the green house. There have been some explants that root and have been successfully transferred to green house. However, copious rooting remains elusive in this genotype.

2. Photobiological regulation of rootstock multiplication: Once the media was formulated we tested the impact of different light wavelengths on G 41 multiplication. It was found that any light intensity higher than 30 micromoles per meter square per second was detrimental for shoot multiplication. Our next goal is to test different light regimes for rootstock micropropagation. The construction of specialized growth chambers has concluded successfully. The impact of light regimes will now be tested. The growth chambers use LEDs that offer a “green” solution to electricity use. LEDs last longer and do not produce much heat.

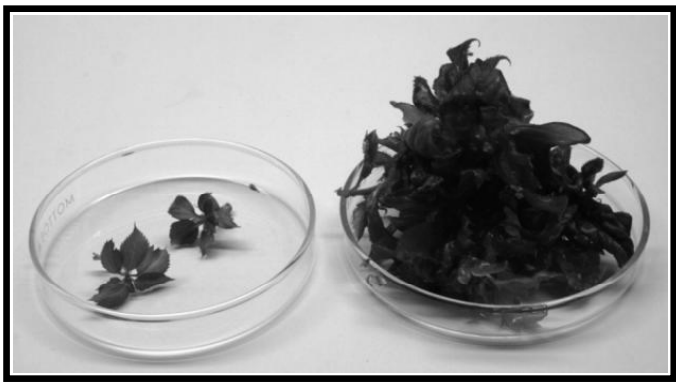
3. Temporary Immersion system.
Standardized media formulation without agar was utilized for temporary immersion system. Figures below illustrate the speed with which the G-41 rootstocks can be multiplied. The graph shows the gain of time in multiplication of the rootstock. A savings of 6 weeks during multiplication is being utilized to develop an efficient rooting system. Second figure shows relative growth of an explant in two weeks in solid media



(left panel) and TIS produced explant (right panel). Last figure shows the magnitude of multiplication if an explant is left in the TIS for four weeks.



With these encouraging results the stage is set to incorporate other troublesome rootstocks or scions in the program. Over the next few months we will test the impact of different wavelengths of light on G-41 multiplication.



ADDITIONAL DEVELOPMENTS

Several undergraduate students have had the opportunity to contribute to this project. Two of them, Danielle Druffel (civil engineering major) and Maureen McFerson (Food Science major) have been awarded a fellowship from CAHNRS at WSU to carry out the research embodied in the objectives of this proposal.

PRESENTATIONS AND PUBLICATIONS

A. Invited Presentations:
1. A Dhingra: Rosaceae Micropropagation and Biotechnology. 4th Acclimatization and Establishment of Micropropagated Plants 2008. Bangalore, India December 2008
2. A Dhingra: Woody Plant Micropropagation. NNII presentation. Yakima, WA December 2008

B. Poster Presentations:
1. S Tariq, M McFerson, S Schaeffer, N Tarly, G Fazio and A Dhingra: Go Forth and Multiply! Establishing an efficient system for G-41 rootstock micropropagation. Annual WSHA meeting, December 2008, Yakima, WA.
2. J Able, E Shay, F Ali Khan, D Druffel, J Cruz, D Kramer and A Dhingra: Building controlled electronic systems to direct plant growth. Annual WSHA meeting, December 2008, Yakima, WA.
3. S Schaeffer, T Koepke, D Jiwan, D Druffel, D DeMars, F Ali Khan, N Tarlyn, T Yang and A Dhingra: Improving tissue culture, micropropagation and biotechnological

approaches in Rosaceae Crops. 4th International Rosaceae Genomics Conference, Pucon Chile March 2008.

EXECUTIVE SUMMARY AND FUTURE DIRECTIONS

Rapid, reliable, cost-effective micropropagation is essential to accelerate progress in genomics, genetic, and breeding (ggb) projects and, fully integrate the emerging biotechnological tools in horticultural crop improvement. Of immediate significance to the Pacific Northwest industry is the scaling up of superior genotypes for rapid commercialization. Several years of progress towards developing a superior genotype is often impeded at the initial step of building liner beds. The infrastructure established with support from this project aims to cater to this very issue.

Future Directions: Encouraged by the current micriprpagation results we have requested for consolidated funding from WTFRC to continue tissue culture based projects in all three major US Crops. The next steps in the project are to test the impact oF LEDs in root growth optimization.