FINAL PROJECT REPORT

Project Title: Rosaceae micropropagation and tissue culture platform

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Cooperators: Tom Auvil, WTFRC; Nnadozie Oraguzie, WSU; Gennaro Fazio, USDA-ARS; Herb Aldwinckle, Cornell, Geneva; Bill Howell, Northwest nursery improvement institute; Todd Einhorn, OSU; Helios Nursery - Tye Fleming and Todd Erickson

Other funding Sources

Agency Name: Helios Nursery
Amount awarded: $15,000
Notes: Funding provided for personnel and operational support in May, 2009

Agency Name: Washington State University
Amount awarded: $60,000
Notes: Funding provided for setting up a 12x10ft walk in growth room to fit tissue culture infrastructure including LED growth chambers and temporary immersion systems

Total project funding request: 30,000

Budget History

Item	**2009**		
Salaries	18,440		
Benefits	7,560		
Wages			
Benefits			
Equipment			
Supplies	2500		
Travel	1500		
Miscellaneous			
Total	**30,000**		

Footnotes:

Note: The term “in vitro” repeatedly used in this project means “in tissue culture”; Magenta boxes are tissue culture vessels made of clear polypropylene material. It is a trademark and does not represent a magenta colored box. LED – Light Emitting Diode; RITA – Temporary immersion system

OBJECTIVES

Major goal of the project: This project addresses the ever-increasing time gap between development of new rootstock or scion genotypes by several breeding programs and their commercial utilization by the growers. This delay represents a financial burden both to the program that develops them and the fruit industry.

The original objectives of the proposal were:

1. Refine or formulate micropropagation protocols for Geneva rootstocks (apple), Pear rootstock OH x F and Polish Quince (Pear)
    - Objective 1A: Finalize the protocol for obtaining rooted rootstocks of G41 and develop similar protocols for G935 apple, OH x F and Pear Quince rootstocks.
    - Objective 1B: Third-party validation of the micropropagation protocols.
    - Objective 1C: Perform a cost-analysis of agar-based or temporary immersion system protocols to assess implementation of the methods in a commercial setting.
2. Define special light conditions for micropropagation of rootstocks and scions.
    - Objective 2A: Identify the most efficient light wavelength combinations for apple and pear rootstocks.
    - Objective 2B: Assess the cost-benefits of utilizing specialized growth chambers in micropropagation.
3. Transition the micropropagation research to the field – sustaining the Rosaceae micropropagation platform.

The objectives were revised as the funding from WTFRC was adjusted and a post-doctoral research scientist could not be hired at the start of the project.

Revised Objectives

Objective 1: Rootstock production: Finalize the protocol for obtaining rooted rootstocks of G41

Objective 2: Rootstock acclimatization: Establish protocols for transfer of material from the lab to the green house

Objective 3: Standardize micropropagation of G-935 (apple), OHXF 87 (pear) and Gisela 6 (Cherry)

SIGNIGFICANT FINDINGS

Apple rootstock:

Geneva 41 rootstock propagation has moved all the way to the greenhouse tests. We have tested first batch of micropropagated plant material in mist beds achieving a survival rate of 65%. These plants were not rooted in the lab but directly transferred to the greenhouse.

Rooting standardized:

We have also worked on resolving the rooting issue with G-41. In tissue culture plants, we can now obtain 100% rooting of explants. Tissue culture media established for G-41 was tested on G-935 micropropagation. The G-935 genotype does not respond well to G-41 media. There is a need for new media formulations for G-935. We have made progress on G-935 micropropagation in agar and are able to achieve 4-6X multiplication on temporary immersion system-based micropropagation.

Pear rootstock:
We have established OHXF 87 micropropagation system both in agar as well as in temporary immersion system. In addition, we have also obtained rooted pear rootstocks.

Cherry rootstock:
Four rootstocks namely Gisela 5, 6, 12 and Krympsk have been successfully established in agar based media. We are able to obtain multiple shoot formation.

RESULTS AND DISCUSSION

Objective 1: Rootstock production: Finalize the protocol for obtaining rooted rootstocks of G41.
Personnel:
Scott Schaeffer – Graduate student funded through an NIH fellowship assisted in project coordination.
Undergraduate students: Salma Tariq, Maureen McFerson, Tammy McGrath, David Rockefeller

An in vitro liner consisting of 4 nodes is placed horizontally on the tissue culture media. After 6-8 weeks when each node develops into one or two individual shoots, the basal liner tissue is excised. Individual shoots are moved to rooting media placed in square transparent boxes called Magenta boxes. The normal light conditions are the conditions used in tissue culture room with 30 micro moles per m2 per sec with 16h day and 8 h dark periods. Under these conditions the rooting media used was supplemented with IBA and no sucrose.

Rooting has been obtained by using modified nutrient salts (MS media) and IBA. Prolific root formation is observed in 4 weeks.

1. Geneva – 41 rootstock micropropagation pipeline is in place. We have established that each new genotype of apple rootstock requires special media formulation. This would explain lack of success in micropropagation in other labs.
A rooted G-41 explant can be obtained in 8-10 weeks with our method. Figure 1 shows a rooted G-41 rootstock after 10 weeks from start of micropropagation.


Figure 1: Rooted G-41 in rockwool placed in liquid rooting media.

2. Geneva – 935 rootstock. As mentioned above, G-935 needs its own media formulation. A derivative media of G-41 has been utilized to multiply G-935 successfully.

The table below summarizes the number of plants currently in the laboratory for G-41 and G-935. All data is tabulated starting August 2009.

Most of the G-41 plants available are in clumps with each clump having at least 3-4 individual shoots. Prior to next subculture, the plants will be divided into individual shoots and placed on media to be stored at 40 deg F for providing chilling requirement. APM is the shoot regeneration media for G-41 and RITA is the temporary immersion system. G-41 is hard to elongate after initiation and most of our efforts have been to devise media or methods to enable elongation of the individual plants.

Date	Step Taken	Total Number of Plants on agar	Amount of time	Multiplication Factor
8/31/2009	Initiation in solid APM	100	1 month	~*3
9/24/2009	elongation in solid APM	293	1 month	
11/5/2009	elongation in solid APM	290	current	

G-41 (11/20/09)	Total plants
Solid APM	290
Rita System	0
Glass Beads	0
Rock Wool	0
	290 clumps

Similarly for G-935 plants where we recently had good success in rapidly multiplying plants in agar, the numbers are tabulated below. RG is the shoot regeneration media and RITA is the temporary immersion system. The individual plants counted here are clumps of 2 or 3 individual shoots. Not all steps listed below are for multiplication.

Date	Step Taken	Total Number of Plants	Amount of time	Multiplication Factor
8/11/2009	Initiation in solid RG	20	1 week	
8/20/2009	Multiplication in Rita (liquid RG media)	40	2 weeks	2X
9/9/2009	liquid RG Starch and sorbitol	40	1 week	0
9/14/2009	Glass beads	40	1 week	Contamination
9/18/2009	Rock Wool	1	Still in rock wool	
8/19/2009	Initiation in solid RG media	20	2 weeks	
8/24/2009	Multiplication in Rita (liquid RG)	40	2 weeks	2X
9/9/909	liquid RG Starch and sorbitol	40	1 week	
9/16/2009	Glass beads	20	1 week	
9/28/2009	Rock Wool	2	Still in rock wool	
9/22/2009	Initiation in solid RG media	20	7 weeks	
10/1/2009	multiplication in RITA (liquid media)	40	2 weeks	2X
10/16/2009	liquid RG starch and sorbitol	80	1 week	
10/23/2009	Glass beads	40	1 week	
11/2/2009	Rock Wool	10	1 week	

10/9/2009	Initiation in solid RG	18	8 weeks
10/22/2009	Multiplication in Rita(liquid RG)	120 (80 plants still in Rita)	2 weeks
11/2/2009	Glass Beads	40	

G-935	Total plants
Solid RG	340
Rita System	80
Glass Beads	40
Rock Wool	15
	475 clumps

Objective 2: Rootstock acclimatization: Establish protocols for transfer of material from the lab to the green house.

One of the major reasons for tissue culture derived plant mortality is sudden drop in relative humidity. To avoid humidity related mortality, the explants will be moved towards rooting while enclosed in Magenta boxes. In cooperation with Tye Fleming and Todd Erickson, multiplied rootstock shoots were moved to mist beds to be tested for survival. It was found that 65% of the plants survived.

Objective 3: Standardize micropropagation of G-935 (apple), OHXF 87 (pear) and Gisela 6 (Cherry)
Personnel:
Tyson Koepke, Derick Jiwan and Chris Hendrickson – Graduate students assisted in project coordination.
Undergraduate students: Noelle Podlich, Ashley Koepke, Matt Allan, Jake Abel, Aaron White, Valeria Lopez-Lozano, Amanda Medina, Christina Duncan and Cory Druffel

The micropropagation of G-935, OHXF 87 and Gisela 5, 6 and 12 is well standardized.

News from other micropropagators: Gennaro Fazio is planning a teleconference to put together a combined teleconference to measure our individual progress on micropropagation of G-41 and other Geneva roots. Currently no other information is available to us regarding what other groups are doing.

*Role of Cooperators***:**

Previous Cooperators: These cooperators were listed for the original project. However, in the currently revised framework we will only work with Tom Auvil and Gennaro Fazio.
Tom Auvil, WTFRC – Coordinate tissue culture activities with the nursery industry and enable acclimatization of tissue culture derived plant material.
Nnadozie Oraguzie, WSU – Identify scions and rootstocks that should be multiplied in vitro to support the breeding program activities.
Gennaro Fazio, USDA-ARS – Implementation of standardized protocol to commercial nurseries.
Herb Aldwinckle, Cornell, Geneva – Validation of protocols established in our laboratory.
Bill Howell, Northwest nursery improvement institute – Supporting the research activities based on micropropagation and utilizing in vitro multiplied rootstocks in orchards.
Todd Einhorn, OSU – Micropropagation of Quince rootstocks.

New cooperators: Tye Felming and Todd Erickson
Tye Fleming and Todd Erickson will utilize in vitro multiplied G-41 rootstocks and help in greenhouse based rooting and acclimatization. We have tested a set of G-41 roots and have been in contact for future transfer of materials.

CAHNRS Undergraduate Research funding:
There are two undergraduate students working on this project under supervision of Amit Dhingra and Scott Schaeffer. Salma Tariq and Maureen McFerson are heading the G-41 and G-935 projects respectively. The project was selected for CAHNRS Undergraduate Research Fellowship and will specifically support establishment of rooting under RBG light spectra. The results were presented at the annual CAHNRS awards banquet on April 4th 2009. The results of this project were presented at the annual Sunrise Orchard Field day.

Executive Summary

Overview: Micropropagation of new rootstocks and scions takes substantial experimentation especially for difficult genotypes. Over the past two years of funding, our program has formulated media specific to some of the desirable rootstocks and scions of apple, pear and cherry. In addition, we have built infrastructure like Temporary immersion systems and LED-growth chambers to accelerate plant growth. We are now set to utilize carbon dioxide in our growth systems to accelerate plant growth even more without the impediment of vitrification and other tissue culture stresses.

Progress: We have over 290 G-41 and 475 G-935 plants. They have not crashed in growth. However, in order to avoid any such issues all the tissue culture plants will be transferred to 40 deg F for a month to provide chilling requirement. We are excited about our success with sweet cherry scion and rootstock progress. A total of 4 rootstocks have been multiplied in agar-based media. In Pears, only OHXF 87 has been tested and successfully multiplied. We have also tied up with the nursery industry to take the methods from the lab to the field. A total of 110 G-41 plants were tested. Additional plants have not been tested as we are in the stage of multiplying more roots. The plants tested in the nursery were derived from agar based media as well as RITA system. There were four treatments that worked for plants multiplied in RITA system. This method needs to be refined further but holds most promise in terms of speed and multiplication capacity.

Future Direction:
Resources: WSU has provided $60,000 for renovation of a freezer into a growth room specifically for supporting our tissue culture activities. Currently we are constrained by space that limits how many roots we can produce. With the new facilities that will not be an issue and we can expand our production capacity.
Funding: With all the methods established we are all set to take the next step. However, there is a need for a full time scientist to assist us in multiplying all roots in large numbers for experimental work. These funds were requested last time but not granted. The funds obtained from WTFRC and Helios Nursery were used to hire several undergraduate students to continue the work on method development and meet the revised objectives. Most of these undergraduate students are enrolled in 18-hour credits at WSU. A post-baccalaureate Physics student was also hired to develop LED-based growth chambers as well. Helios Nursery funding arrived when we were four months into the project and it was impossible to hire a post-doc for 8 months.

Plan for sustaining the micropropagation platform:
The methods developed in the laboratory are the property of WSU. We are very eager to share these with willing partners. The University is extremely flexible in any arrangements we want to make. Our vision is to utilize these methods to provide a rapid multiplication system to our industry. In return a revenue stream of 5 cents per every plant sold can come back to the program to sustain future research. To realize this potential we require at least three years of committed funding to support a scientist and undergraduate students with some operation funds.