FINAL PROJECT REPORT

Project Title: Accelerating pear breeding progress with early-flowering plants

PI: Richard L. Bell
Organization: USDA, ARS
Telephone: 304-725-3451 Ext. 353
Email: richard.bell@ars.usda.gov
Address: 2217 Wiltshire Road
Address 2: Appalachian Fruit Research Station
City/State/Zip: Kearneysville, WV 25430

Cooperators: None

Total Project Request: **Year 1:** $14,308

Other funding sources: None

Budget 1
Organization Name: USDA, ARS **Contract Administrator: Ingrid Charlton**
Telephone: 215-233-6554 **Email address: Ingrid.charlton@ars.usda.gov**

Item	**2013**
Salaries	
Benefits	
Wages	$10,490
Benefits	$ 840
Equipment	
Supplies	$ 2978
Travel	
Miscellaneous	
Plot Fees	
Total	$14,308

OBJECTIVES

The objectives were to transform 'Bartlett' pear scion cultivar and 'Old Home' x Farmingdale' pear rootstocks with *PtFT1* and *BpMADS4* genes. Following selection and verification of transgenic clones, tree architecture and flowering were to be evaluated in the greenhouse. Flowering clones were to be artificially pollinated with cross-compatible parents and fruit and seed development, objective measures of fruit quality (size, % soluble solids, titratable acidity), and seed yield would be evaluated. Finally, one clone of 'Bartlett' and one clone of OHF97 would be selected for future studies of horticultural practices to minimize the generation cycle and maximize fruit/seed production.

SIGNIFICANT FINDINGS

Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97,OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets.

RESULTS AND DISCUSSION

Stock shoot tip cultures were multiplied to provide sufficient leaves for transformation/plantlet regeneration experiments. Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97,OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets. An experiment has commenced to transform these cultivars with the *BpMADS4* gene. Additional experiments are underway to enhance our regeneration/transformation success. [Changes in the standard protocol/medium composition were made by my technician without my knowledge, which may have led to reduced plantlet regeneration/transformation.]

Work on this project was delayed because the funding was not available until August 20, 2013, and hiring the Biological Aide candidate required another 3 weeks.

EXECUTIVE SUMMARY

In order to respond efficiently to present and future breeding priorities and challenges, accelerated strategies are needed to more rapidly transfer important genes from less than optimum germplasm resources into commercially feasible genetic backgrounds. Development of this technology would be particulary important to deal with potential challenges imposed by climatic factors and water availability. Genetic transformation with genes which control flowering, such as *PtFT1*from poplar and *BpMADS4* from birch offer one technology to reduce the non-flowering period to one year, substantially reducing the generation cycle. This technology has been successfully applied to apple using *BpMADS4* and is being developed for plum and pear with *PtFT1* at our research station. This project seeks to develop the genetically transformed early-flowering plants and greenhouse production system to allow rapid cycling breeding for pear which will enhance incorporation of valuable traits from unadapted pears into commercial varieties.

Summary of findings

Several experiments were conducted at two-week intervals to genetically transform 'Bartlett', OHF97, OHF87 and 'Conference' with the *PtFT1* gene. All leaf explants of the uninoculated controls produced callus, with no necrosis (leaf death), but the percentage of regeneration was low (9%). Callus was produced on non-necrotic leaf explants for 'Bartlett' (92%), 'Conference' (91%), OHF97 (65%) and OHF87 (35%). No new adventitious shoots, however, were produced which survived selection on the kanamycin antibiotic-containing tissue culture medium. Changes in the protocol have been instituted to increase the efficiency of plantlet regeneration and recovery of transgenic plantlets.

Future directions

Experiments will be conducted to increase the efficiency of the tissue culture-based plantlet regeneration and transformation system. Work planned for the coming year includes continued transformation of 'Bartlett' and 'Old Home' x 'Farmingdale' 97 pear rootstock with *PtFT1* and *BpMADS4*. 'Conference', a pear cultivar with a high adventitioius regeneration capacity, will also be transformed. Transgenic clones generated with be evaluated in the greenhouse for tree architecture and flowering. Flowering clones will be pollinated with cross-compatible parents and fruit development, objective measures of fruit quality (size, % soluble solids, titratable acidity), and seed yield will be assessed. Single clones of 'Bartlett' or 'Conference' and one clone of OHF97 will be selected for future studies of horticultural practices to minimize the generation cycle and maximize fruit/seed production.