FINAL PROJECT REPORT

Project Title: Factors Affecting Meristem Fate in Rosaceae

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Cooperators:

Total project funding request: Year 1: 24,403 Year 2: 22,460 Year 3: $0

Other funding Sources

Agency Name: **WSU**
Amount awarded: **$ 50,000**
Notes: For genomics related equipment directly relevant to this project.
Budget data provided in "Other funding sources" is for informational purposes only, and for WTFRC to understand the scope of the project. These estimated costs are not presented as formal cost-sharing and therefore do not constitute a cost-share obligations on the part of WSU. Moreover, there is no requirement for WSU to document this other support of project as part of any cost-share or matching obligation.

Total Project Funding: 46,863
Budget History - Pullman

Item	2007	2008	
Salaries			
Benefits			
Wages	2,500	3,000	
Benefits	288	345	
Equipment			
Supplies	12,500	10,000	
Travel	2,000	2,000	
Miscellaneous[1]	5,000	5,000	
Total	22,288	20,345	

Footnotes:
1 Miscellaneous – funds requested for sequencing

Budget History - Prosser

Item	2007	2008	
Salaries			
Benefits			
Wages	1,000	1,000	
Benefits	115	115	
Equipment			
Supplies			
Travel	1,000	1,000	
Miscellaneous			
Total	2,115	2,115	

Footnotes:

NOTE: This proposal was originally funded by the Technology Commission and later transferred to Cherry Commission. Therefore the timing for the Final Report on this proposal has arrived a few months in advance.

OBJECTIVES

Flower numbers in cherry determine overall fruit quality. Rootstocks have a major impact in determining the fate of the meristem as it transitions from a vegetative to a reproductive stage. This project was aimed at identifying the gene-level contribution of rootstocks towards the transition and consequently the flower number. For sweet cherry production, this is one of the important traits that controls flower set and understanding of this phenomenon is expected to inform us regarding thinning practices to obtain best quality fruit. Starting early May 2007 (period of floral bud initiation), entire 1-year-old spurs (fruiting wood in subsequent season) were collected from same-age trees for analyses. The samples were collected until December 2007 when the buds reach dormancy based based on the previous research carried out by Matt Whiting.

Briefly, the specific objectives and the progress made are listed below:

1. Generation of cDNA or EST (Expressed sequenced tag) libraries from floral tissues derived from specific scion and rootstock combinations.

2. The ESTs will be sequenced and made available as a community resource.

3. Cloning of the flower meristem identity genes from the EST libraries derived from different genotypes.

4. Quantification of gene expression levels for these genes at transcriptional level using real time-PCR and/or northern blot analysis.

(Please see glossary of technical terms used in this report)

SIGNIFICANT FINDINGS

1. Type of rootstock (Gisela or Mazzard) impacts the expression of flowering-related genes in the bud spurs of the same scion (e.g. Bing). What this means is that the DNA level variation in these genes can be potentially utilized for developing molecular markers to regulate crop load in new varieties.

2. Bud spurs are an important site where the rootstock effects manifest themselves. Our study has the potential of identifying additional genetic factors that may impact other flower or fruit related traits.

RESULTS AND DISCUSSION

1. Generation of cDNA (complementary DNA) libraries from floral tissues derived from the scion and rootstock combinations

RNA derived from DNA is used for developing cDNA libraries. We have established an economical and streamlined RNA extraction method from spur tissues, which is being prepared for a peer-reviewed publication. RNA yields are generally very low owing to the nature of the tissue. There were no previously published protocols for RNA extraction from this type of tissue. We have successfully prepared 8 cDNA pools from tissues collected in May and September 2007 representing

Bing/Mazzard, Bing/Gisela, Rainier/Mazzard and Rainier/Gisela. Capturing of cDNAs in a library is underway. This resource can be utilized in the breeding program activities for identifying important genes related to other desirable traits.

2. The ESTs will be sequenced and made available as a community resource

ESTs are unique short sequenced parts of cDNA. Generally the way EST libraries are made full length transcripts are not cloned. This generates a computational problem. No wonder there are 300,000 ESTs for apple in the database but only 30-40,000 genes are predicted to be present in the apple genome. Since the RNA isolation has been standardized, we have successfully generated cDNA pools and are now employing next generation sequencing technologies to sequence the cDNAs en masse. All the data analysis will be performed in the laboratory and will be available in a searchable database in four months. As in case of objective 1, the sequences derived from this objective will serve the needs of the breeding program to develop molecular markers for desirable traits.

3. Cloning of the flower meristem identity genes from the EST libraries derived from different genotypes.

Several genes out of the ones involved in transition of meristematic tissue from vegetative to reproductive stage namely, Apetala1 (AP1), Flowering Locus T (FT), Constans (CO), Leafy (LFY), Terminal Flower 1 (TFL1), Cauliflower (CAL) and Frigida (FRI) were amplified. The sequencing of these DNA fragments is currently underway. Comparisons of the sequences with those from Apple and Peach will provide insight into probable gene function. Once the nucleotide sequence is deciphered, computer based comparisons will enable identification of any nucleotide differences in these genes. This is an important piece of information required for the breeding activities.

4. Quantification of gene expression levels for these genes at transcriptional level using real time-PCR and/or northern blot analysis.

This work is currently underway. We have been able to use RNA to amplify the flowering-related genes in sweet cherry. Now we have to fine-tune the method for doing quantitative analysis. All the samples and RNA are available. We will be testing the relative expression of flowering-related genes in different scion/rootstock combinations. Based on these differences, nucleotide-level variations will be identified as in case of objective 3 and information provided to the sweet cherry breeder, Dr. Nnadozie Oraguzie for developing molecular markers for this useful trait.

ADDITIONAL DEVELOPMENTS

Leveraged Funding:
1. Graduate Student Support: This project is being carried out by Tyson Koepke who is a graduate student in the Dhingra Lab. Tyson is pursuing his graduate studies under the Molecular Plant Sciences Program that has been ranked 2nd in the nation recently. This proposal has been accepted for NIH Protein Biotechnology Graduate Training Program that provides Tyson 2 years of complete support for his Ph.D. work. That amounts to $ 70,000 for two years.

2. Equipment Grants: We have been able to leverage another $ 650,000 in equipment funds from the college and the department to enable genomics-related experiments that will directly benefit this project. Equipment includes Genome Sequencer, a high sensitivity spectrophotometer to accurately measure RNA and DNA, a freezer mill to grind hard tissue like the bud spurs and Bioanalyzer for RNA quality control.

PRESENTATIONS AND PUBLICATIONS

1. T Koepke, MD Whiting and A Dhingra. Discovery of Genomic Factors Regulating Flower Density in Sweet Cherry. Washington State Horticultural Association 103rd[d] Annual meeting, Wenatchee, WA. December 2007

2. T Koepke, MD Whiting and A Dhingra. Factors affecting meristem fate in Rosaceae. Oral presentation at the Annual Molecular Plant Science Retreat. Pullman, WA February 2008

3. T Koepke, MD Whiting and A Dhingra. Identification of Genomic Factors Regulating Flower Density in Sweet Cherry. 4[th] Rosaceae Genomics Conference, Pucon, Chile. March 2008

4. A Dhingra, MD Whiting and T Koepke. Using genomics tools to understand rootstock-induced floral bud initiation in Rosaceae. Oral presentation at the 9th International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Geneva, NY. August 2008. To be published in Acta Hort proceedings.

5. T Koepke, MD Whiting and A Dhingra: Streamlined protocol for isolation of RNA from woody perennial tissues for transcriptome analysis. Under preparation for Plant Methods (peer-reviewed)

EXECUTIVE SUMMARY AND FUTURE DIRECTIONS

This proposal is very unique as it attempts to understand the impact of rootstock on specific physical organs in the scion. The composite nature of the sweet cherry is biologically unique compared to other model systems where impact of roots has been studied on flowering. The findings from this project will have a major impact on our understanding of this phenomenon in apples and pears as well. While we are looking at a specific subset of genes involved in meristem transition, our approach is poised to identify all other genes that may play a role in other traits like fruit set, pollination or even fruit quality. Since the inception of this project, the technological infrastructure in our lab for genomics research has become cutting edge. What that means is that instead of just looking at a few genes at a time, we now have the capacity to study the entire complement of genes that are being impacted by the rootstocks in the same scion. The genome sequencer has the capacity to read the frequency of every gene's occurrence and thus provide a quantitative value on the expression of each gene.

Thus far, in our experiments we have confirmed that expression of certain meristem identity genes in one scion is impacted by the type of rootstock they are grafted on. This preliminary dataset has formed the foundational data for a federal grant proposal that we will submit to USDA-NRI in February 2009. The proposal is already prepared and is the research topic for the graduate student Tyson Koepke working on this project. Funding will be requested to extend this work to identification of rootstock induced phenomenon and characterize nucleotide-level polymorphisms for molecular marker development.

This research has been presented at national and international forums and has been very well received. A preliminary part of the research is being encapsulated into a peer-reviewed publication and a conference proceeding paper.

Glossary of technical terms used in this report

Molecular Markers: These are DNA sequences like a bar-code that are linked to a desirable trait like red fruit peel. The markers find use in a breeding program to screen or bar-code progeny at the seedling stage for the desirable trait. Seedling without the bar-code can be eliminated in the green house saving space and resources.

cDNA and ESTs- complementary DNA and Expressed Sequence Tags: Genes in a plant cell are made of DNA that represents the structure. The functional derivative of DNA is RNA. In the lab we can capture the RNA and derive DNA (called complementary DNA) from it again and read the nucleotide composition. It is not possible to read RNA as it degrades very fast. Short pieces of this RNA-derived DNA pieces represent the functional aspect of DNA and is termed as the expressed sequence tag.

Real-time PCR: PCR is a method wherein a single molecule of DNA is multiplied or amplified to over a billion molecules by a chain reaction. When PCR is performed to quantify the initial number of starting DNA molecules in a given sample at a set time point, it is termed real-time PCR. It informs scientists of the functional output of any given gene.