FINAL PROJECT REPORT

Project Title: Cherry genome project

PI:	Amit Dhingra	**Co-PI**:	Matthew Whiting
Organization:	Washington State University	**Organization**:	Washington State Univ
Telephone:	509-335-3625	**Telephone**:	509-786-9260
Email:	adhingra@wsu.edu	**Email**:	mdwhiting@wsu.edu
Address:	Johnson 46	Address:	24106 N. Bunn Rd
City:	Pullman	City:	Prosser
State/Zip:	WA 99164	State/Zip:	99350

Co-PI:	Nnadozie Oraguzie
Organization:	Washington State University
Telephone:	509 786 9271
Email:	noraguzie@wsu.edu
Address:	24106 N. Bunn Rd
City:	Prosser
State/Zip:	WA 99350

Cooperators: Ananth Kalyanaraman, WSU; Herman Silva, Lee Meisel, Chile; Cameron Peace, WSU; Amy Iezzoni, MSU; Todd Einhorn, OSU

Other funding sources

Agency Name: USDA – CSREES Specialty Crops Research Initiative
Amt. awarded: $3.8 million plus equal matching, Sep 2009 – Aug 2013
Notes: "A total systems approach to developing a sustainable, stem-free sweet cherry production, processing and marketing system". PIs – Whiting, Dhingra and Oraguzie.

Agency Name: USDA – CSREES Specialty Crops Research Initiative
Amt. awarded: $7.2 million plus equal matching, Sep 2009 – Aug 2014
Notes: RosBREED. The cherry genome data will serve as a scaffold for identifying SNPs using various sequencing technologies. PIs – Iezzoni, Peace and Oraguzie. Collaborator – Dhingra.

Agency Name: USDA – CSREES National Research Initiative
Amt. awarded: $224,000
Notes: Scaffold sequencing and data de-convolution method development to assemble the apple genome. An Apple Genome Sequencing Initiative. PIs – Dhingra and Kalyanaraman.

Agency Name: Universidad Andrés Bello (Herman Silva and Lee Meisel)
Amt. awarded: $27,000
Notes: Collaborative arrangement with Dhingra on the cherry genome and transcriptome project.

Agency Name: Dhingra and Oraguzie Start-up funds
Amt. awarded: $30,000
Notes: Obtained the initial data on cherry genome sequences

Agency Name: Roche Inc.
Amount awarded: **$30,000**
Notes: Funds being used at 454 to generate scaffold DNA libraries and sequencing to enable efficient assembly of the genome.

Total Project Funding: $48,000

Budget History:

Item	**2010**		
Salary			
Benefits			
Wages			
Benefits			
Supplies	44,000		
Travel	4,000		
Miscellaneous			
Total	**48,000**		

ORIGINAL OBJECTIVE

1. Develop a Genomic BAC library: Entire sweet cherry genome or DNA from Stella (self-fertile progenitor of many new varieties) will be captured in manageable parts of small size using established methods (with Amplicon Express as collaborators).

2. Establish a scaffold to reconstruct the sweet cherry genome: By utilizing a proprietary method of sequencing using techniques developed for apple genome, rapidly generate a scaffold to reconstruct the sweet cherry genome.

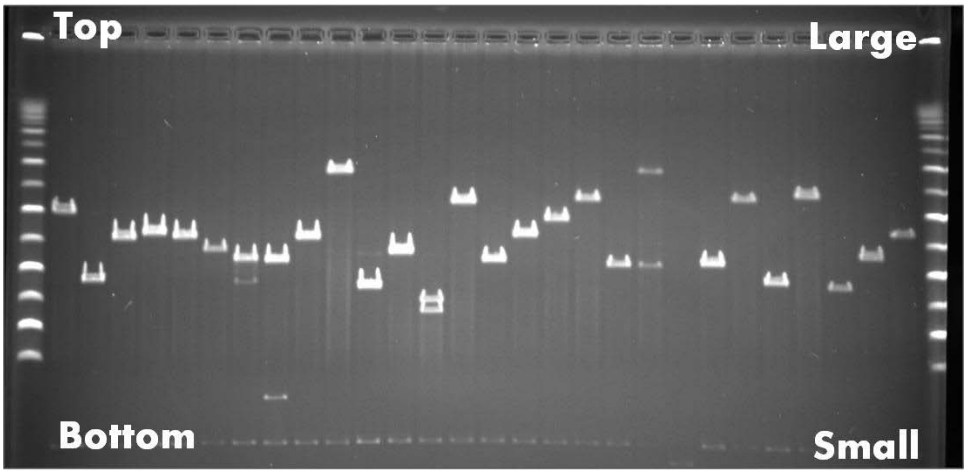
SIGNIFICANT FINDINGS

Objective 1: BAC Library

Definition: BAC Library – It is a method of capturing the large mass of the genome or DNA from any cell and breaking it into small pieces. This needs to be done so that the genome can be studied in manageable pieces.

The basic aim of this project was to expand on the information already generated with funding from Dhingra, Oraguzie and the Chile group and physical resources for cherry genome research in our lab. Support from this project enabled us to construct a high quality BAC DNA library that represents a collection of large pieces of genomic DNA captured in a way that we can multiply them individually, thus making it easy to work with sections of the genome. DNA is made of four letters, A, T, G and C called bases. The average size of the genomic section in the library is 120,000 bases (Fig. 1). The library consist of over 20,000 such pieces representing the genome over 5 times.

Figure 1: This image represents a hard proof to scientists that we have successfully captured the genomic DNA from sweet cherry. The figure is that of a gel in which DNA is separated based on its size. The smaller DNA is present near the bottom of the gel.



The BAC library was prepared as scheduled by June 2010.

Objective 2: Scaffold Sequencing

We had proposed to accomplish this by December 2010. However, to enable this aspect of the project the DNA from over 20,000 BAC fragments needs to be isolated and pooled. This work is underway at Amplicon Express and we expect to commence sequencing in January. The timeline to obtaining all data and final assembly will thus be completed between April – June 2011.

RESULTS & DISCUSSION

To summarize, the BAC library is ready and is currently being processed to move ahead with Objective 2 of performing sequencing. The second step will enable us to generate a scaffold on which genomic sequences can be "hung" to generate the map of the genome.

Impact of Sequence Information: Having a genome sequence does not instantly produce a new variety or solution that the farmers can implement directly in their fields in the coming season. It is the foundational information that once established will be used from here on to serve as a basis of physiological solutions in the near-term and novel varieties in the longer-term.

The cherry GGB group envisions that the genome sequence will be utilized in three broad applications for cherry improvement.

1. Physiological solutions – Near term
Target – Existing varieties and New varieties
All physiological processes in plants are under the control of genes. Prior to the utilization of genome sequence for this purpose, it is important to understand which gene or set of genes controls a desirable process. For example, to develop mechanically harvestable cherry there is a need to understand which genes respond to ethephon and make a certain variety mechanically harvestable. This work is currently ongoing in the program supported by the USDA-SCRI grant to Whiting, Dhingra and Oraguzie.

We are in the process of identifying genes related to the abscission process specifically at the junction of fruit and its pedicel. The cherry genome sequence available so far has been screened for potential genes and the differences in these genes across ethephon responsive and non-responsive varieties. This information is expected to be utilized in finding varieties that will respond to ethephon and the gene-based differences will serve as molecular markers to develop new varieties that can be hand-picked or mechanically harvested depending on the needs and capacities of the farmer.

2. Controlled Sport induction, CSI – Mid-term and Long-term
Target – Existing varieties
In collaboration with Dorrie Main's program, we have functionally tested a gene from Peach that provides broad spectrum and long-term resistance to powdery mildew. Same gene is present in sweet cherry as well. We found that by comparing the peach gene with sweet cherry genome. This highlights another utility of the sweet cherry genome. The only caveat to utilizing this gene is that, resistance is observed when the gene becomes non-functional due to a mutation. In our program we have developed a method where we can regenerate thousands of same variety clones using leaf material. This platform can be used to create sports in existing varieties. How many dollars can be saved if Rainer and Bing were naturally resistant to powdery mildew? This project is not funded however; we are working with Nnadozie Oraguzie to explore the possibility of generating such sports.

A long-term advantage of having the sports is that they can be utilized as parents in future sweet cherry crosses. Since the mutation will be known it can be easily tracked using molecular methods.

3. Novel variety development using molecular markers – Near and long term:
Target: New varieties
As the scientific community discovers which genes control what traits or information from other plant systems becomes available, molecular markers can be rapidly generated to be utilized for parental screening. One such example is the ACS/ACO gene in sweet cherry. The ACS gene has a deletion in cherry compared to the gene in apple. This feature has been utilized to develop a marker by Cameron Peace's group. Now the question is if that deletion is absent in some cherry variety. If yes, then does that cherry respond to ethylene? Answering such questions with the help of genome information can enable rapid development of desired varieties for the PNW cherry industry.

The significance of genome information will far outlive the duration of this project. Each economically important trait or desirable quality in the fruit tree is controlled at some level by genes. An accessible genomic blueprint of cherry enables us to pinpoint what gene or group of genes are responsible for agriculturally important traits. This information will guide cherry improvement in both the short and long term future. Another testimony to this fact is that scientists have now discovered the gene underlying skin and lung cancer in humans utilizing human genome information. As in the case of humans, the potential economic benefits to the industry are apparent. With the cherry genome sequence in hand, we can develop unique varieties for the PNW combining all priority traits that can create lucrative economic opportunities ranging from production to post-harvest stages.

BROADER IMPACTS

Presentations: The cherry genome information has been highlighted at several forums over the last year including WSHA meetings. In 2009, the PI was invited to speak at the Hort Show about Enabling Economic Resilience through Genomics Research. Besides that, the work has been shown as poster presentations at annual international meetings like American Society of Plant Biology and Plant and Animal Genome Meeting. The cherry genome will be presented at the 5th Rosaceae Genome Conference in South Africa in 2010.

Training opportunities: This project has been steered by graduate student Tyson Koepke in collaboration with Artemus Harper, a computer scientist in the program supported by USDA-NRI.

EXECUTIVE SUMMARY

Significant progress: The objective of generating a BAC library from sweet cherry has been completed. The second objective of generating scaffolds for the genome is in progress and we expect that it will be completed by June 2011. Collaborations with Roche Inc. and Andres Bello University and our start-up funds have provided extra resources to the tune of $87,000 to develop a much finer assembly of the cherry genome.

Outcomes and summary of finding: Preliminary cherry genome sequence data are available that are being used by our program to identify coordinates and sequence information of important genes linked to desirable traits like abscission (Dhingra) and ethylene responsiveness (Peace). In summary this is just the start of the most efficient way of connecting traits to genes, an emphasis of our fruit crop genomics program.

Future directions: Abscission-related genes will be tested in sweet cherry parental selections and breeding population to ascertain which varieties has the potential of being mechanically harvestable. We have one proposal under review at NSF, and others at various stages of writing to build upon this foundational information. Our programmatic approach is to connect traits with genes using function information. Future projects are aimed at applying this approach in new and novel ways to the improvement of sweet cherry.