FINAL PROJECT REPORT

Project Title: Gene discovery & controlled sport induction (CSI) for pear improvement

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Total project funding request: Year 1: 54,300 **Year 2:** 59,492 **Year 3:** 63,252

Budget History

Item	Year 1: 2007/08	Year 2: 2008/09	Year 3: 2009/10
Salaries	30,000	31,200	32,448
Benefits	12,300	12,792	13,304
Wages			
Benefits			
Equipment			
Supplies	10,000	11,000	13,000
Travel	2,000	2,000	2,000
Sequencing		2,500	2,500
Miscellaneous			
Total	54,300	59,492	63,252

Pear is an important PNW crop as well as an important member of the Rosaceae family. However, at the beginning of this project, pear genomic resources were scarce. This project was aimed at setting up a platform to identify a gene or set of genes underlying important physiological problems. This knowledge has application in today's pear orchards as well as it can serve as a foundation for future pear improvement via breeding. Recognizing the fact that there is only one pear breeding program in the US and that improvement via breeding can take several decades, the second aspect of this proposal was aimed at establishing a platform that will help in rapid improvement via sport induction that is not random but targeted. For the success of the second activity, we first need to identify trait-gene relationship. Therefore the two major objectives of the proposal were highly complementary.

At the onset of this project two very important traits that affect pear consumption and its production were short-listed. First one was improvement of *post-harvest quality* and storage abilities of pear varieties grown in the Pacific Northwest and second longer term goal was to fulfill the urgent need to develop a *dwarfing pear rootstock.*

ORIGINAL OBJECTIVES: Proposed objectives of the project were:

1. Prioritization of economically important pear traits.
2. Gene discovery for establishing trait-gene relationships using an economical yet high-throughput methodology called Differential Display
3. Controlled Sport Induction using tissue culture derived propagules combined with high-throughput screening of allelic diversity for genes responsible for desirable trait.

SIGNIFICANT FINDINGS

Objective 1: Prioritization of economically important pear traits.

After constant feedback from the industry over the past three year, it was found that the priority traits for pear improvement have not changed much since the late George Ing published an article in Acta Hort in 1993. It was intriguing why similar issues remain. Partly it is due to the fact that pear varieties remain the same, they have a long generation time and another that scientists have always attempted at using solutions generated for apple in pear. As is apparent, pears are a different organism and some information can be borrowed from apples however, pear-specific research will be urgently needed to resolve pear-specific issues.

Pears are not apples and apples are not pear.
Although their family they may share…..

After several industry visits and discussions with growers and packers, one important issue is lack of consistency of the product that reaches the shelf. Not much effort has been made to understand the underlying genomic or genetic reason for most physiological disorders. The solutions for several disorders have brought the industry into a profitable entity however for further progress, effort will have to be focused on understanding how the existing pear trees respond to chemical treatments, how to handle pears like pears and not modify apple processing lines and suffer nearly 30% loss due to scuffing or post-harvest damage.

The pear enterprise can be divided into three parts: production, processing and post-harvest stages. Each of these stages requires minor adjustments to reduce the losses that the industry has to face. These issues have become the cornerstone of a larger, team-based and interdisciplinary proposal

submitted to the NW Pear Bureau. We would like to take these issues to the USDA-SCRI or NIFA proposal this year too.

2**.** Gene discovery for establishing trait-gene relationships using an economical yet high-throughput methodology called Differential Display.

A comparative gene profiling between Bartlett and D'Anjou fruit peel and core has rapidly yielded information about genes that control several quality aspects of the fruit. The first and the one of the important ones we have identified and continue to work on is a gene that is the proposed cold-induced ripening gene. This gene has not been described in the past and is expected us to enable develop effective ripening strategies for pear. Also, it would serve as a target for controlled sport induction in Bartlett pear to change its shelf life.

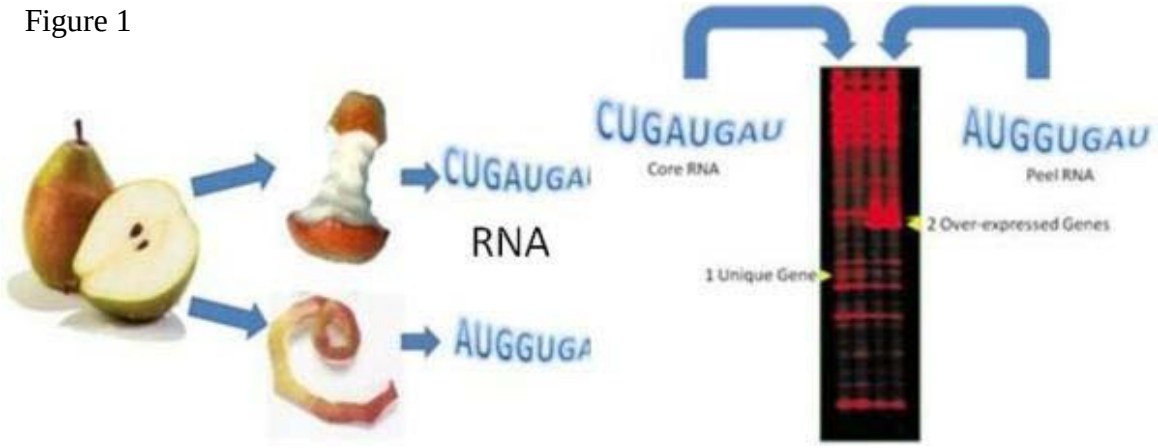
3. Controlled Sport Induction using tissue culture derived propagules combined with high-throughput screening of allelic diversity for genes responsible for desirable trait.

We have established the method of generating new plants from leaf material from Bartlett and D'Anjou pear. The methods for generating targeted mutations are being refined for pear. In addition, we have perfected the techniques for rootstock micropropagation that can enable rapid multiplication of any sport scion that is generated through our CSI approach.

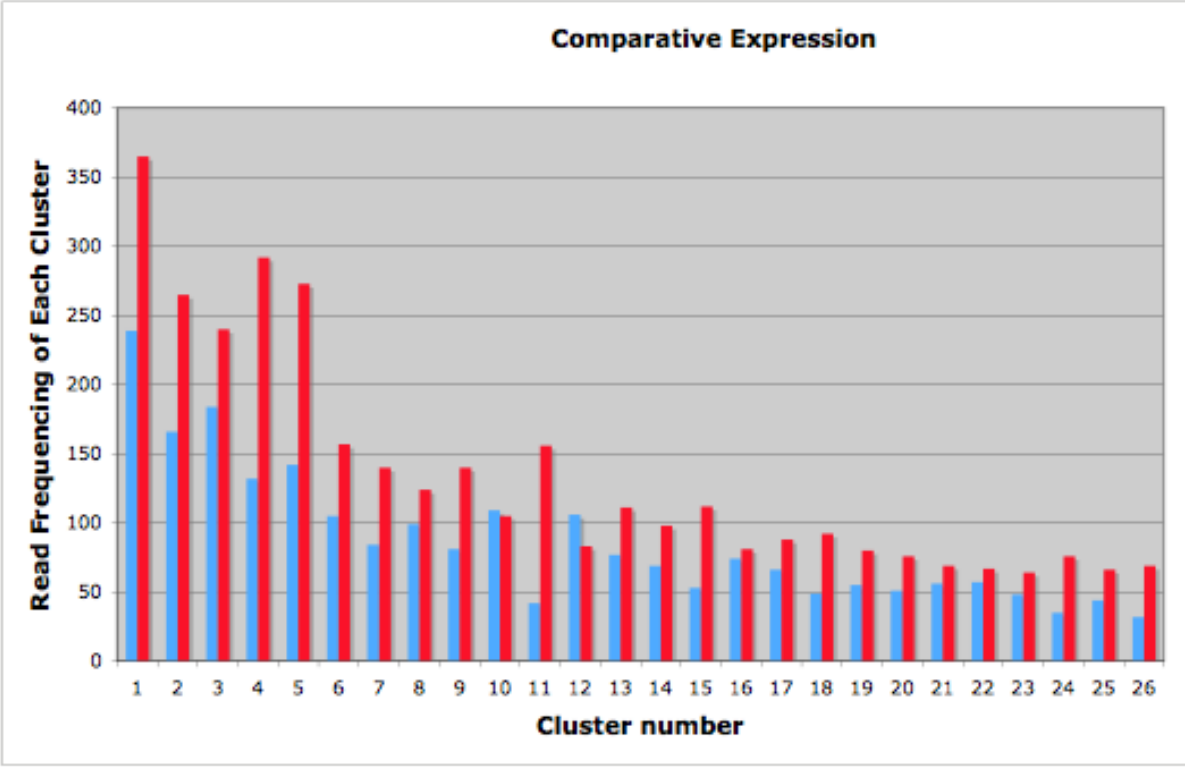
RESULTS AND DISCUSSION

The methods employed in gene discovery in pear are depicted in Figure 1. Peel and core samples were taken from fruit sterilized with ethanol. These samples were transferred in liquid nitrogen for return to the laboratory. By grinding samples in the Spex SamplePrep 6870 freezer mill we were able to obtain high quality RNA ready for analysis.

Figure 1



RNA was isolated from the ground tissue using a Qiagen RNA extraction kit. We performed differential display experiment as previously planned. Besides the gel based differential display, we have performed comparative RNA profiling using the 454 next-generation sequencer. It provides sequence based information on genes and represents the entire transcriptome at the same time. In short we can capture the response of the entire transcriptome in one shot. The graph on next page shows such a snapshot where a cluster represents a single gene and read frequency indicates its abundance. The bars in blue and red represent two different genotypes. The data was obtained from core and peel tissues of Bartlett and D'Anjou harvested at a comparative developmental stage.



By applying custom-developed computational script we analyzed this data for identification of novel differentially expressed genes in the tissue. This revealed the differential expression of numerous unknown genes, as expected in such a relatively uncharacterized organism. However, we discovered a differentially expressed gene (termed *Pyrus communis* membrane-integral protein, or PcMIP). Further computational analysis of this gene shows that it likely serves as a signal receptor, transmitting the cold signal in winter pear. This gene was found 8 times in D'Anjou peel tissue only, not in Bartlett. A review of literature related to this gene suggests a role in ethylene signaling and regulation. Based on this computational and literature analysis, we hypothesize this newly discovered gene in pear to regulate the 2nd-stage ethylene biosynthetic burst, and subsequent ripening in pear. Recent work by Sugar (OSU), Mitcham (UC-Davis), and Kupferman (WSU) support this model by revealing that exogenous ethylene application can circumvent this proposed regulatory mechanism. This gene will serve as a target for our CSI approach and as a powerful molecular marker in pear research and breeding efforts, allowing for rapid crop improvement.

CSI experiments: Although suspension cultures have been established for Bartlett and D'Anjou we have incorporated a unique concept of targeted mutation induction using leaf material. This will be performed with the gene gun and is going to be more rapid than the radiation process. We have two initial targets - reduction of juvenility and non-browning. Some of these mutants can be directly utilized as new varieties or in the breeding program. The mutations are induced by transient introduction of

DNA-RNA hybrid molecules as shown in figure on previous page. DNA bases in dark have been modified to induce mutations.

For development of callus, we have tested several parameters as outlined in Figure below.

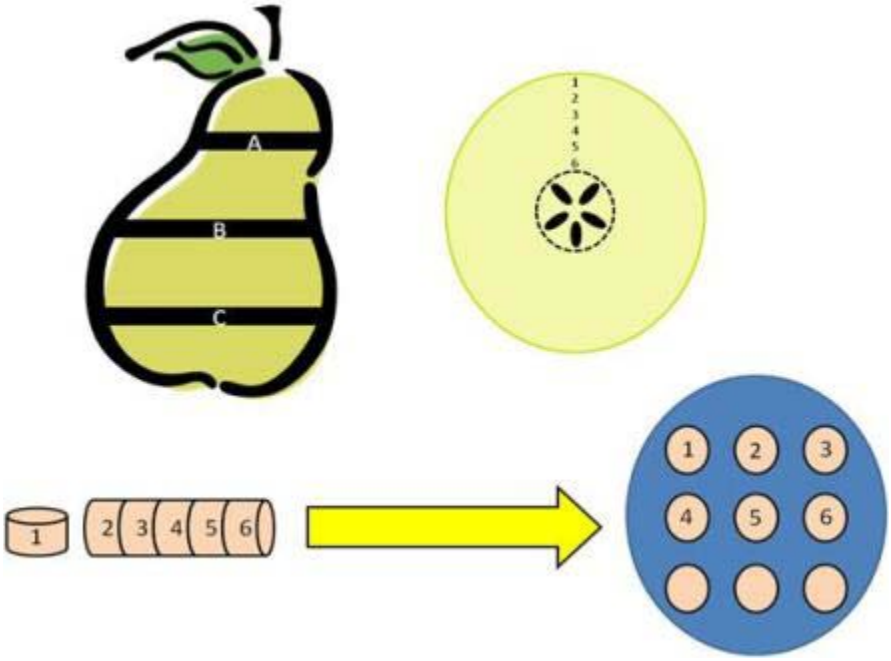


Figure on pear callus production depicts the procedures employed to assess the productivity of callus formation by various parts of the pear fruit. Cores were taken from each of three selected parts of the pear; the top (A), middle (B), and bottom (C). The samples cores were cut into multiple sections and discs were cut from each section. Callus was able to grow from sections A, B, and C with no section showing any significant increased callus growth. While callus was derived from nearly all tissue, tissue nearest to the core (6 and 5) generally displayed the highest ability to grow callus. Optimal growth of callus was determined to occur by changing the SH media every three weeks.

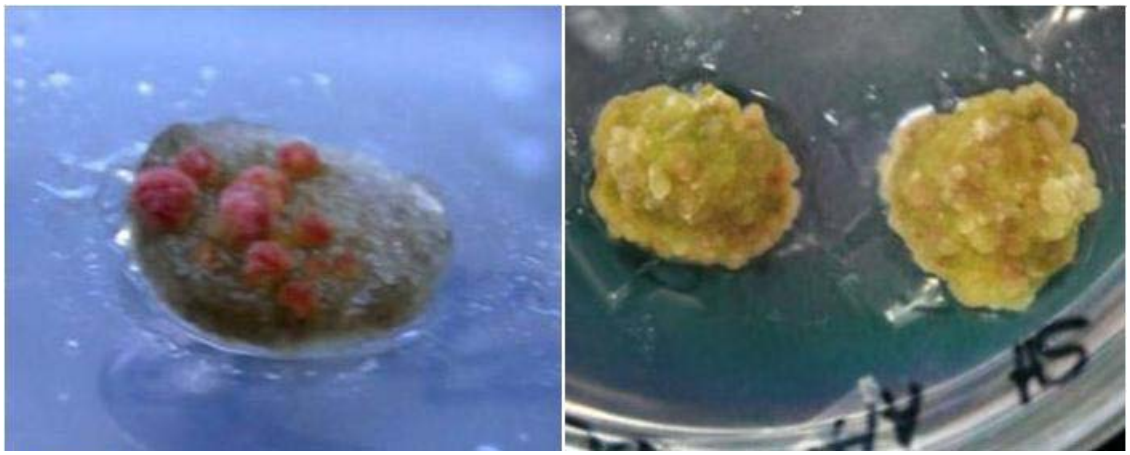


Figure above displays early callus growth (left) on pear tissue discs after two weeks of growth and a later stage of callus growth (right) after two months of growth. **Right panel** displays cellular growth after 40 days of inoculation. After sufficient callus was produced, callus tissue was transferred into liquid media. Cells were shaken to produce individual callus cells.

Outreach:

1. The work and the ideas underlying this project were featured in the invited presentation at the USApple annual convention in August 2007 to communicate the concepts to the stake holders. It was also features at the WSHA invited presentation in 2009.
2. The work was presented in an invited talk at the AEMP 2007 meeting in Portugal in September 2007 and AEMP 2008 in India.
3. The preliminary concepts were presented at the WSHA meeting in 2007, 2008 and 2009 by Scott Schaeffer and Chris Hendrickson graduate students in the Dhingra lab.
4. This work was presented at the Annual Rosaceae Genomics Conference in Chile in March 2008 and American Society of Plant Biologist annual meeting in July 2008 and 2009.

EXECUTIVE SUMMARY

Significant Progress and outcomes and summary of findings

Some of the major steps we have accomplished to sustain the pear improvement efforts are to have established a strong feedback mechanism from the industry, established a strong community network of pear researchers worldwide and established genomic resources previously missing in the community. We have identified traits where we can implement the knowledge of genomics today for improving the existing pear orchards. Discovery of the putative cold-induced ripening gene in D'Anjou is a major accomplishment without years of phenotyping on diverse genetic material. We have also established methods for generating new plants from leaf tissue of scion varieties and micropropagation rootstock material. This accomplishment will aid in developing novel pear varieties using controlled sport induction.

Gene discovery is essential to identify the factors responsible for Pacific Northwest pear traits and can be exploited to improve the local economy's influence in domestic and international markets. Due to the narrow germplasm present in pears, a non-traditional program such as the Controlled Sport Induction method can be used exploit this knowledge to introduce new traits to existing varieties. New pear varieties could be developed to address immediate problems in the pear industry such as storage time and dwarfing as well as less immediate traits such as texture and color.

Controlled Sport Induction is becoming a realistic goal for improvement of pear traits. Samples of Bartlett and D'Anjou pear have been collected for the gene identification project. We have currently been successful in establishing a proficient RNA extraction technique in fruits.

New varieties of pear can be tested commercially after the complete procedures of this technology are worked out. As this approach involves no transgenic modification, there will not be any issues with implementing this technology. During mutagenesis (sport induction) some deleterious mutations may also be generated, but can be eliminated in the segregating population. The clonal variants will also serve as defined donors or parents of desirable traits for Marker Assisted Breeding. Materials developed using this technology may offer opportunities for new intellectual property in the form of novel clonal variants.

Future directions

We plan to further characterize other genes that we have already identified as differentially expressed between two fruit types. Some of these genes are directly related to fruit quality traits. The results and methods established in this proposal are serving as a basis for a larger proposal submitted to NW Pear Bureau. The funding will be utilized for a larger USDA-SCRI proposal. Since gene discovery and its characterization is a basic research component, we are submitting a proposal to NSF to understand the ripening mechanism in further detail.