FINAL PROJECT REPORT

Project Title: Cultivar improvement via controlled sport induction (CSI)

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Total project funding request: Year 1: 26,150 Year 2: 36,725 Year 3: $0

Other Funding Sources - none

Total Project Funding: 62,875

Budget History

<table>
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<tr>
<th>Item</th>
<th>2007</th>
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<tr>
<td>Salaries</td>
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<tr>
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<td>Benefits</td>
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<td>Miscellaneous</td>
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<tr>
<td>Total</td>
<td>26,150</td>
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Footnotes:
OBJECTIVES

Naturally occurring sports have been a source of improvement to apple cultivars in the past but it is a chance process that is long-term and unpredictable. We had proposed a procedure to accelerate this natural process of sport induction and regulate it by targeting economically important traits. As cause and effect or gene-trait relationships continue to be established both in our program and by other programs, the scope of this platform will expand to encompass numerous important traits.

Proposed objectives of the project were:

1. Using tissue culture, establish cultures from selected apple cultivars
2. Standardize the technique for efficient sport induction
3. Perform a pilot experiment with tissue culture material for identifying allelic diversity for the genes that regulate fruit firmness (ACC-synthase and ACC-oxidase)

SIGNIFICANT FINDINGS

1. Leaf material from all apple cultivars tested is better for rapid regeneration of new plants. Regeneration from fruit derived cell lines were extremely slow in regeneration that can result in large number of somaclonal variants.

2. Leaf size and light intensity are highly critical for shoot regeneration from leaf material in M26, Gala, Pinova apple cultivars.

RESULTS AND DISCUSSION

Leaf regeneration has been optimized for Royal Gala, Pinova, and M26 leaves as seen in the figure. M26 leaves produced large amounts of regenerants on N6MS media supplemented with BAP and NAA. Royal Gala and Pinova, however, were unable to produce many regenerants on this media. Instead, regenerants were copiously produced on N6MS media containing TDZ, IBA and BAP.
Apple leaves ranging from 2 to 5 cm were selected from healthy looking plants. These leaves were cut into 1cm strips and placed upon N6MS media. Plates were placed in a dark room temperature cabinet for a week. After dark incubation, plates were transferred to a 75 deg F light chamber with light intensities of 15 to 100 μmol per meter square per second. The leaves were kept under these conditions for three to four weeks and the numbers of generated shoots were compared.

The initial N6MS media contained BAP and NAA. If plants did not generate from leaves on this media, new leaves were tested under the same condition with varying concentrations of IBA, TDZ, and BAP.

Controlled Sports Induction or CSI (general schematic above) is being performed using the regeneration system developed in the lab. Leaf segments are bombarded using a gene gun. This instrument introduces thousands of small gold particles covered in RNA/DNA hybrid oligonucleotides called GRONs (Gene Repair Oligonucleotides) into the leaf cells. The GRONs target specific genes for mutation and produce a protrusion in the leaf’s genomic DNA. This protrusion tricks the plant into “repairing” its DNA, thus mutating it’s own DNA in a specific manner. In the end no extraneous DNA is added into the plant’s genome. Thus this is not a transgenic method and does not produce GMOs.

We have targeted the ACS genes. In addition, PPO gene responsible for flesh browning, ALS gene, a mutation in which causes herbicide resistance, TFL gene that reduces juvenility in apple have been targeted. The plants are being regenerated to identify mutants at the present stage. Several regenerants are in the process of being screened.

**ADDITIONAL DEVELOPMENTS**

1. Graduate Student Support: This project is being carried out by Scott Schaeffer who was a lab manager for a year and has recently enrolled into the graduate program in the Dhingra Lab. Scott 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 Scott with 2 years of complete support for his Ph.D. work. That amounts to $70,000 for two years.
2. Equipment Grants: Research proposed in this project has been supported further by the procurement of a gene gun that obviates the need for radiation-based mutagenesis. The gene gun is worth $25,000.

3. Undergraduate training: Four undergraduate students have been trained in this project so far providing them with a unique opportunity to get involved in horticultural plant research.

**PRESENTATION AND PUBLICATIONS**

A. Invited Presentations:


B. Poster Presentations:


EXECUTIVE SUMMARY AND FUTURE DIRECTIONS

The aim of the proposal was to establish a rapid and efficient method of creating controlled sports in a given variety. Several successful varieties suffer from major production or storage issues. As we establish cause and effect relationship between trait and genes with gene discovery and apple genome project, this platform will be increasingly utilized to remove the shortcoming in a variety. Mutations in ethylene genes for fruit firmness, PPO gene for reducing flesh browning, ALS gene for herbicide resistance and TFL gene for reducing juvenility are being generated. Some of these regenerants can be utilized as source of novel traits in the breeding program as well. The outcome of this exercise will be mutated, non-transgenic plants. The improved clonal cultivars can be tested commercially and used directly as this approach involves no transgenic modification. During mutagenesis (sport induction) other mutations, some deleterious, may also be generated but those 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. The data generated from the activities mentioned above will be leveraged to attract long-term federal funding for continued apple improvement.

Future Directions: This work is not being submitted for renewal this year. Our future aim is to utilize this platform to develop targeted mutations on a contract basis from interested groups. This project will be sustained from such a revenue source. In addition, the projects being submitted to NSF and USDA in the coming year will benefit from the infrastructure develop with support from this project.