FINAL PROJECT REPORT

Project Title: Chemical Genomics

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Cooperators:

Other funding Sources

Agency Name: No
Amount requested/awarded:
Notes:

Total Project Funding: 60,000

Budget History:

No money left.

RECAP ORIGINAL OBJECTIVES:

In this collaborative project, we proposed to apply a chemical genomics approach to rosaceous crops, and help solve some of the problems facing the Washington tree fruit industry. One of the major issues is how to improve fruit size and fruit quality. It has been well documented that fruit development and ripening are regulated by plant hormones such as auxin, gibberellins, and ethylene. For sweet cherry (*Prunus avitum*), we will focus on the effect of gibberellic acid (GA) on fruit size and quality, as well as tree size.

The plant hormone gibberellin has long been known to modulate development throughout the plant life cycle. Mutants that are impaired in GA biosynthesis or response tend to have small dark green leaves and reduced stem length. Thus understanding the regulatory mechanisms of GA could help to produce dwarf crops. GA mutants are also often defective in seed germination and floral development, and are delayed in flowering time (Fleet and Sun, 2005). In cherry, GA application is currently used by growers worldwide for improving fruit quality and delaying maturity (Lenahan et al., 2006; Maib et al., 1996). Vigorous shoot growth in sweet cherry trees can also be controlled with gibberellin-biosynthesis inhibitors such as such as prohexadione-Ca (Manriquez et al., 2004).

Our specific objectives were:

1. To screen the available chemical libraries and identify the chemical compounds which affect the GA pathway,
2. To study the effect of selected chemicals on gene expression and identify the marker genes involved in fruit development, ripening, and tree size using subtraction cloning and microarray technologies,
3. To study the effect of the chemical compounds on fruit shelf life, and quality, as well as tree size.
4. To train Washington State students in the cutting-edge discipline of chemical genomics.

SIGNIFICANT FINDINGS

1. Screened a 100,000 chemical library using strawberry and Arabidopsis.

2. 252 and 165 chemicals have been isolated from Arabidopsis and strawberry screenings, respectively. Among them, 125 chemicals exhibit the similar effects on both Arabidopsis and strawberry.

3. Of 125 chemicals, 77 have inhibitory effects, and 48 have stimulatory effects.

4. Twenty-five chemicals were selected for large scale field test in Bing in Prosser, WA, 2007. These chemicals were chosen because they showed best effects on seed germinations in both Arabidopsis and strawberry.

5. Several chemicals were effective in controlling skin color, flesh color immediately after application.

6. These chemicals affected the buds per spur and flower numbers per bud in following season.

7. Six chemicals were further selected for large scale field test in Pullman, WA, 2008. Selection of these chemicals was based on their performance in the field test of year 2007. The chemicals affected the fruit size, and fruit color, which were consistent to the results in Prosser, WA, 2007.

8. In conclusion, we have identified a few very effective chemicals which control fruit color and flower numbers.

RESULTS & DISCUSSION

In last report, we indicated that 25 selected chemicals were used to spray in Prosser orchard on May 30, 2007. The normal spray with GA3 was used as control. Each chemical was sprayed on the cherries in a branch of one tree. The experiment was repeated twice in two different trees. The cherries were harvested on June 22. We further analyzed the cherry weight, skin color, flesh color, firmness and Brix.

As shown in Figure 1-4, the 25 compounds had a variety of impacts on the traits we measured as compared to control. The most obvious effects were the skin color and flesh color which are desirable traits for consumers, while they did not show significant changes on the fruit firmness.

In 2008, we selected 6 chemicals for a large scale field test. These chemicals were selected based on their performance (positive and negative effects) in 2007 test. Since the Prosser orchard had no many fruits because of the bad weather this spring, we did the field test this year in Tukey Orchard, Pullman, WA in July 2008. We also changed the sweet cherry variety from Bing to Rainier in order to observe the color effects clearly. Two independent trees were used for all treatments.

Figure 5 shows that six chemicals can be separated into two groups, negative group (No. 2, 3) and positive group (No. 1, 4, 5, 6) based on their effects on the fruit weight. They all increased fruit color as compared with GA control. As for fruit firmness, No. 4, 5, 6 had no significant difference as compared with GA control. Among six chemicals, No. 4 showed the best in all measurements. Figure 6 are the photos exhibiting the effects of No. 4 chemicals on fruit ripening. In the same tree, the fruits sprayed with No. 4 chemical were ripen a week to 10 days later than no spray fruits in the same tree. The fruit weight in sprayed fruits was significantly improved (~40% increase). It also had better effects on fruit weight, skin color than GA control. However, the fruit firmness was comparable with GA control. We made efforts on isolation of RNAs from cherry fruits treated with different chemicals, but the quality of RNA was not very good to proceed the subtraction cloning.

In conclusion, we have identified a few powerful chemicals which affect sweet cherry fruit quality and flower numbers. The tests on different locations and different varieties in different years indicate that these chemicals are more effective than GA. These chemicals may also have the potential for other tree fruits such as apple and pear.

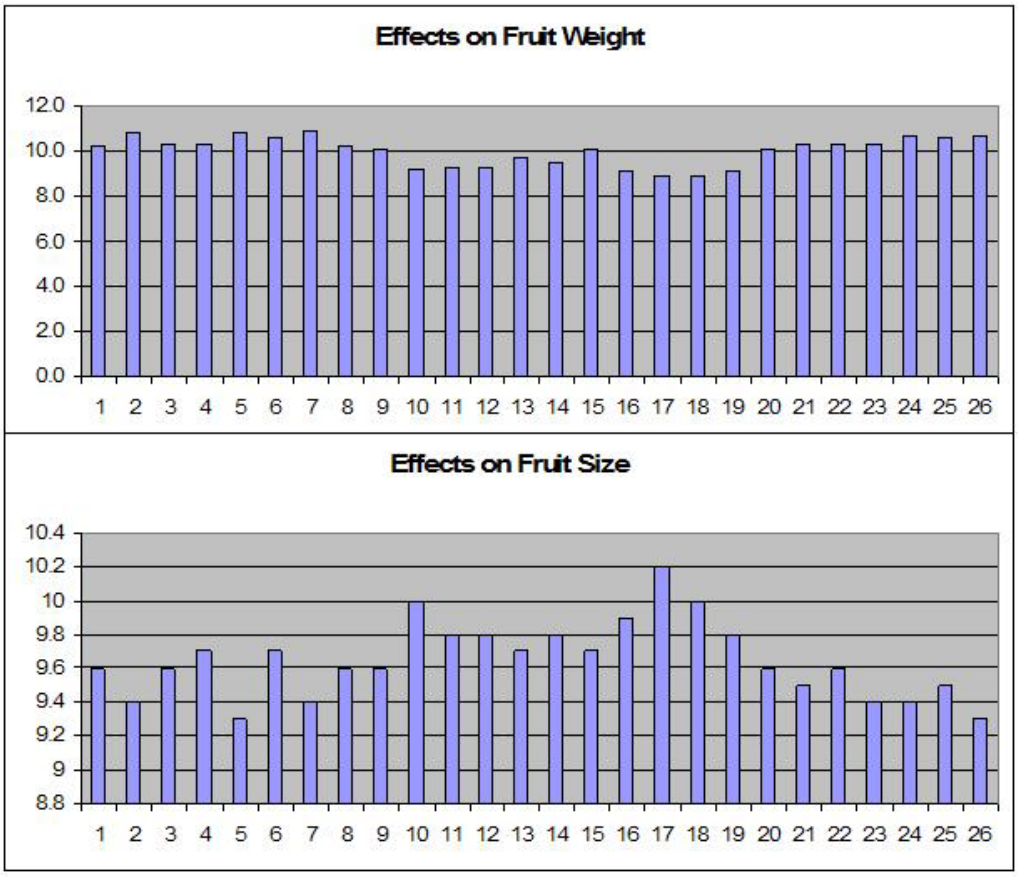

Figure 1. The effect of 25 selected compounds on the fruit weight and size. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

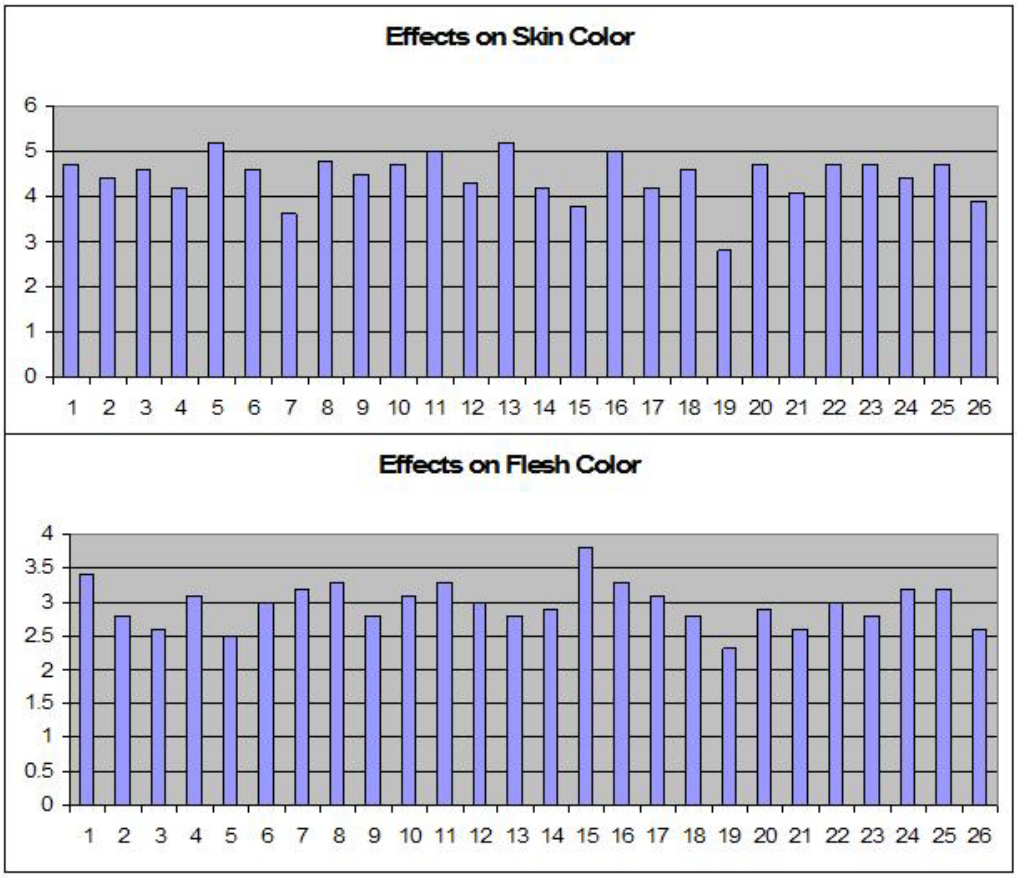

Figure 2. The effect of 25 selected compounds on skin color and flesh color. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

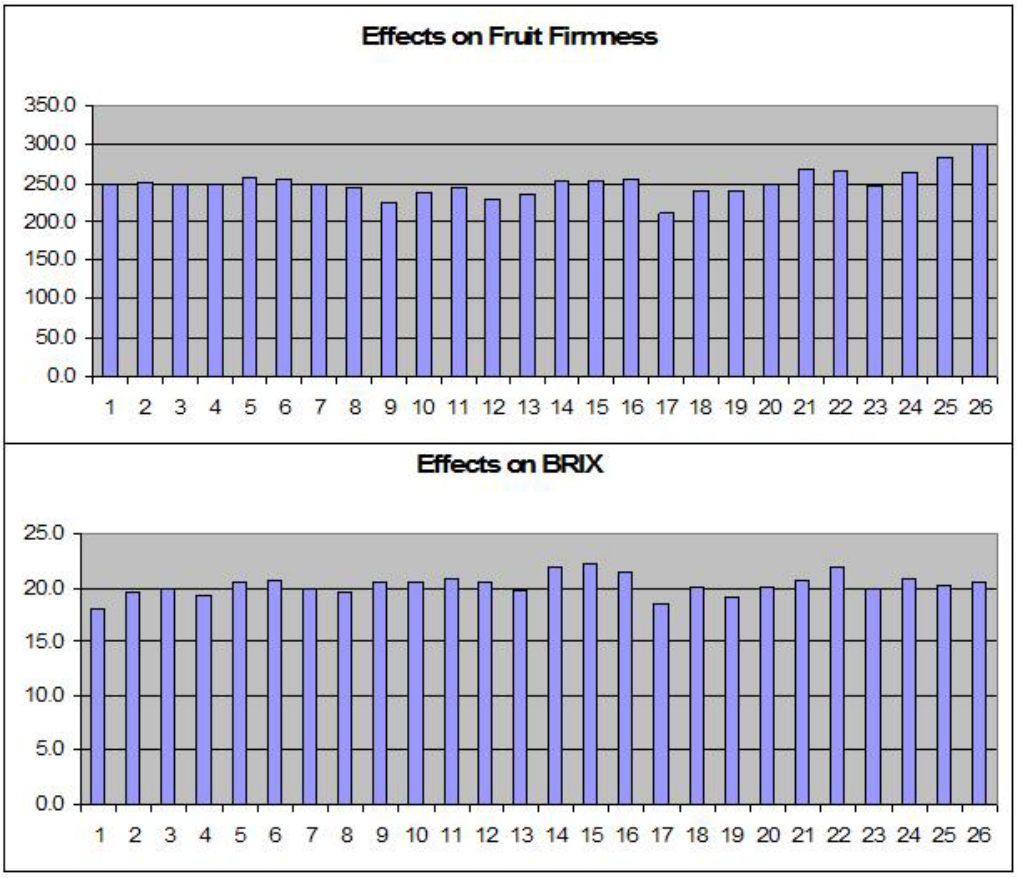

Figure 3. The effect of 25 selected compounds on fruit firmness and Brix. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

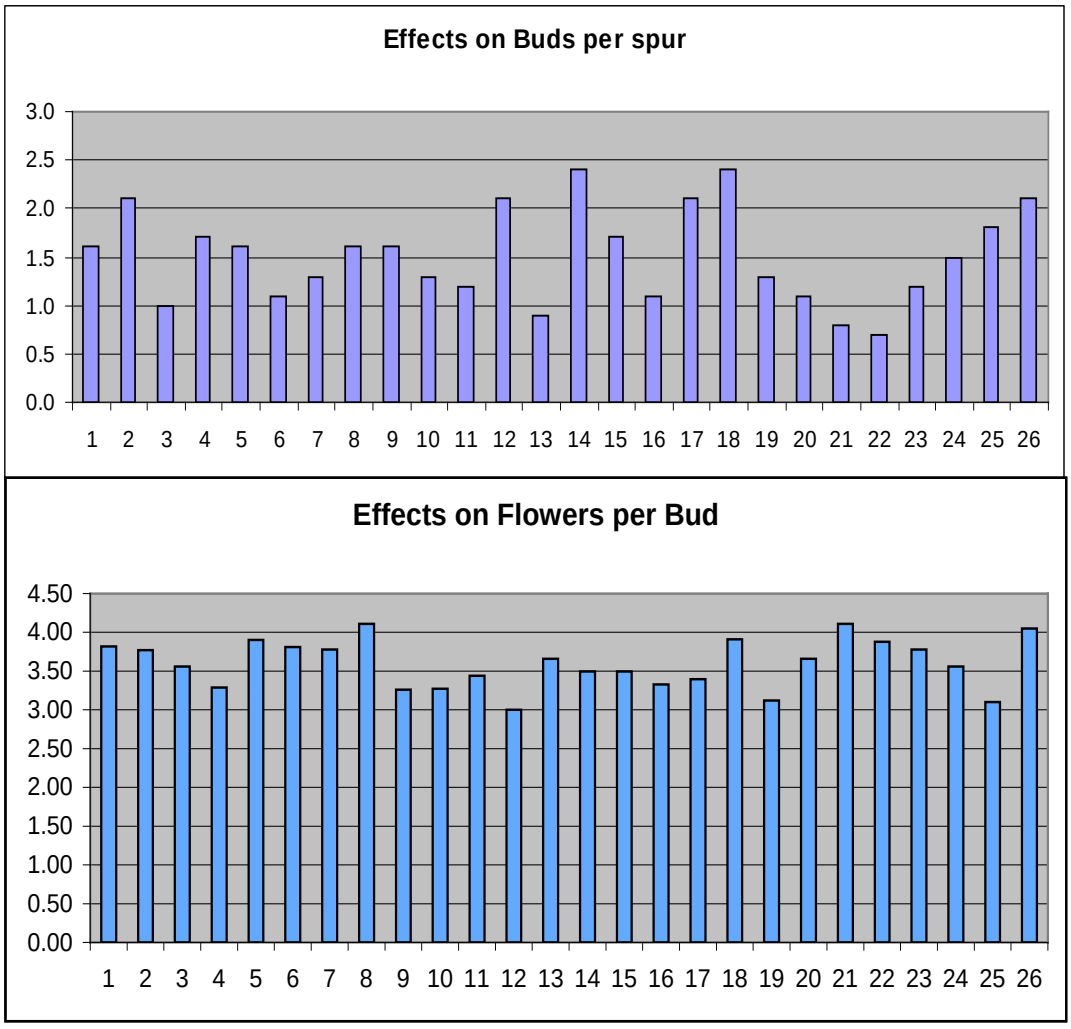

Figure 4. The effect of 25 selected compounds on bud numbers and flower numbers. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

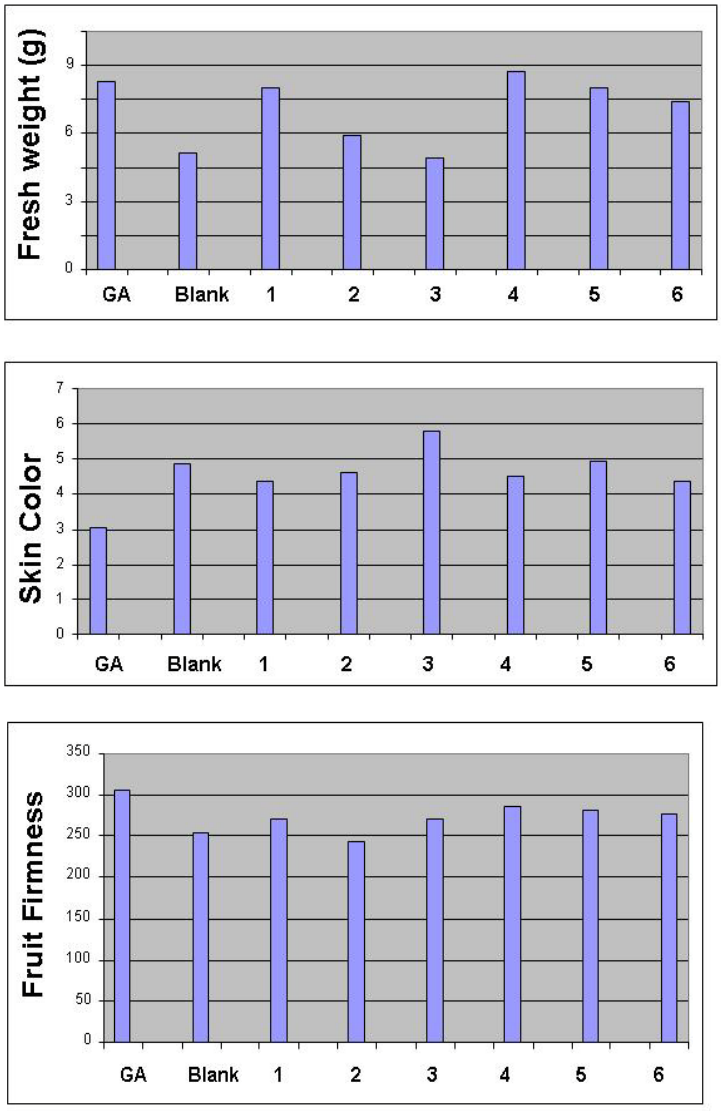

Figure 5. The effect of 6 selected compounds on the fruit weight, fruit size and fruit firmness. (Pullman, WA, 2008)

Figure 6. The effect of a small molecule (No. 4) on sweet cherry fruit ripening and fruit size. The photos show the fruits with treated and untreated from the same tree. This chemical can delay the fruit ripening and increase the fruit size. (Pullman, WA, 2008)

EXECUTIVE SUMMARY

Chemical genomics is a new high-throughput approach for determining gene function using small bioactive molecules to activate/inactivate gene products (i.e., proteins). Recently, chemical genomics has been used to better elucidate hormonal signaling in Arabidopsis. In this report we summarize our use of a chemical genomics approach for sweet cherry improvement.

From screening a 100,000 format chemical library, we identified more than 100 bioactive molecules that affect (elicitors and inhibitors) the gibberellin pathway. Twenty-five of these were applied to fruiting sweet cherry limbs in the field. We observed a variety of effects on fruit color, firmness, soluble solids, and weight. Furthermore, several compounds inhibited floral bud initiation and show potential as crop load management tools. A larger scale test using 6 selected chemicals in different location and different variety showed the similar results.

To sum up, we have identified a few very powerful chemicals which affect sweet cherry fruit quality and flower numbers. These chemicals are more effective than GA. Besides, these chemicals which are effective in sweet cherry may work in other tree fruits such as apple and pear, too. Our results indicate that using chemical genomics approach can save time and money for tree fruits gene disco very and crop improvement.