

BIOAG PROJECT FINAL REPORT

TITLE: UNDERSTANDING THE MOLECULAR BASIS OF PHYTONUTRIENT COMPOSITION DIFFERENCES IN ORGANIC TOMATOES VIA QUANTITATIVE TRANSCRIPTOME ANALYSIS

PRINCIPAL INVESTIGATOR(S) AND COOPERATOR(S):

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ABSTRACT

In a previous BIOAg supported study, it was shown that tomatoes grown with organic nitrogen sources had higher phytonutrient contents than those fertilized with readily available nitrogen. It is hypothesized that improved nitrogen cycling may be the underlying cause of these observed differences. In addition, defense compounds are enhanced in tomatoes grown with organic fertilizers, whereas herbivory may enhance them in tomatoes grown with inorganic fertilizers. Fruit and leaf samples from tomatoes grown with organic and readily available nitrogen sources and phenotyped in the preceding study were used to generate comprehensive transcriptome data. This project addresses the priority area of breeding, varietal selection, management practices, processing, or marketing practices to increase food quality, nutrition, safety or access. In this project we successfully performed a high-level transcriptome analysis. The information presented here reflects the quantity and amplitude of the genome level changes associated with each transcriptome compared to specific treatments and further analysis will be needed to understand which specific genes or networks are modulated. However, it is exciting to note that the two soil management regimes elicit differential genomic response from a single genotype.

PROJECT DESCRIPTION

Consistent increases in phytonutrients (antioxidants, phenolic compounds, carotenoids, vitamin C, etc.) in organically grown apple, strawberry, and tomato fruits compared to their conventionally grown counterparts that utilized synthetic fertilizers and pesticides has been reported previously (Peck et al. 2005, Reganold et al. 2010, unpublished data). This led to the hypothesis that organic soil fertility management, which favors a slower rate of biological release of available nitrogen to plant roots, results in greater allocation of photosynthetically derived resources to the synthesis of secondary metabolites, such as phenolics and other antioxidants, than to plant growth. This hypothesis was tested on tomato plants. Overall yield and average fruit size were larger in conventionally (CON) grown plants, but fruit from organically (ORG) grown had greater dry matter and soluble solids concentration. Ripe ORG fruit also had higher concentrations of phenolic compounds, lycopene, and vitamin C than CON fruits, all of which contribute to pest- and stress-related defense responses. Leaf C:N ratios were higher in ORG plants, thus favoring the synthesis of C-based compounds, like phenolics, ascorbic acid, and carotenoids. Preliminary analysis of the transcriptome data revealed that in ORG grown plants expression of certain genomic loci was ramped up. Initial investigation of these differences needed to focus on three major defense-related metabolic pathways (ascorbic acid, carotenoid, and phenylpropanoid). Observed differential responses by specific plant parts at different developmental stages under different production regimes leads to a hypothesis that there is a dynamic relationship between rhizosphere

microbiology and plant roots resulting in altered global gene expression patterns. These alterations in gene expression networks promote allocation of photosynthetically derived resources to the synthesis of defense-related phytochemicals.

Gene expression information can be a valuable indicator of plant's metabolic performance under different production systems. In a study comparing tomato plants grown in either a legume or black-plastic mulch system, plants growing in the legume mulch showed delayed senescence and greater disease resistance than plants grown in black plastic mulch (Kumar et al. 2004). The legume mulch resulted in the accumulation of certain amino acids, vitamins, and organic acids, with a simultaneous decrease in sugars in ripe fruit (Neelam et al. 2008). Plants grown in legume mulch showed increased expression of defense- and stress-resistant genes and genes responsible for nitrogen assimilation and carbon metabolism. These studies studied the expression of known candidate genes and preceded the release of tomato genome thereby being biased in their selection of target genes. The dataset we have already generated utilizes the power of de novo transcriptome analysis to identify global gene-specific responses to the imposed physiological state in our experiments. This will allow us to not only analyze the behavior of previously reported genes, but, more importantly, capture genome-wide and species-specific responses of the metabolic network in their entirety.

The overarching goal of this interdisciplinary project and subsequent research is to determine the physiological and genetic basis that often result in the production of more nutrient dense crops in organic farming systems. This will enable organic farmers and farmers interested in sustainable practices to develop soil fertility and pest management systems that favor the production of more nutrient dense crops. It will also enable plant breeders to focus on developing both more pest resistant and nutrient dense crop cultivars. These nutrient dense foods can contribute to lower caloric consumption, which is one of the main causes of obesity among children and adults in the U.S.

NOTE: These were the originally proposed objectives.

1. Comprehensive transcriptome analysis to identify differentially expressed genes involved in phenotyped metabolic pathways. (Dhingra, Year 1)
2. Perform preliminary wet-lab validation of differentially expressed genes identified in objective 1. (Dhingra, Year 1)
3. Perform statistical correlations between observed phenotypes and gene expression observations. (Andrews, Dhingra, Year 1)
4. Global gene expression analysis to identify differentially expressed gene networks. (Dhingra, Year 1)

We are grateful for the support however reduced funding (\$7000 out of the requested \$39,180) severely crippled the possibility of performing high resolution analyses of the data. While no clear instructions were provided on revising the objectives, we were able to utilize the available funds to address objectives 1 and 4 partially.

OUTPUTS

- Work Completed:

Fruit and Leaf Total Transcriptome Comparisons between Conventional and Organic Treatments and Non-aphid and Aphid Challenged Plants

Gene expression comparisons at greater than 5 fold differences between conventional and organic treatments indicate the organic treatment, while expressing 11% more (expressed sequence tags) ESTs than the conventional treatment, had 33% more ESTs in the over 5 fold increased expression category

than that of the conventional treatment (Table 1 and Figure 1A). The aphid stressed plants, aphid challenged versus non-aphid challenged, had 37% less total expressed transcripts and only 7% of ESTs higher expressed at 5 or greater fold than the non-aphid stressed plants (Table 1 and Figure 1D). Both the conventional/organic and non-aphid/aphid challenged had nearly identical non-differentially expressed numbers of ESTs with the non-differential conventional/organic transcriptomes expressing 107,465 ESTs and the non-aphid/aphid transcriptomes expressing 107,834 non-differential ESTs and were only different by 0.4% (Table1). *Complete transcriptome comparisons indicate conventional treatments elicit more transcriptional diversity with lower expression rates compared to the organic treatment expression profile of fewer, more directed expressed genes. This is a critical observation which necessitates deeper data analysis and validation to establish molecular evidence that organic treatments are utilized differentially by a plant.* The aphid challenged transcriptome points to a less energetic transcription profile compared to the non-aphid challenged transcriptome conceivably due to the loss of nutrients by aphid feeding.

Table 1. Fruit and leaf combined transcriptome conventional/organic and non-aphid/aphid challenged greater than 5 fold EST numbers.

Conventional Treatment/Organic Treatment				
ESTs Higher in Conventional	58,124	ESTs >5x Conventional	936	
ESTs Higher in Organic	51,678	ESTs >5x Organic	1,406	
Total ESTs	109,807	Non-differential ESTs	107,465	
Non-Aphid/Aphid Treatment				
ESTs Higher in Non-Aphid	66,312	ESTs >5x Non-Aphid	1,843	
ESTs Higher in Aphid	41,653	ESTs >5x Aphid	130	
Total ESTs	109,807	Non-differential ESTs	107,834	

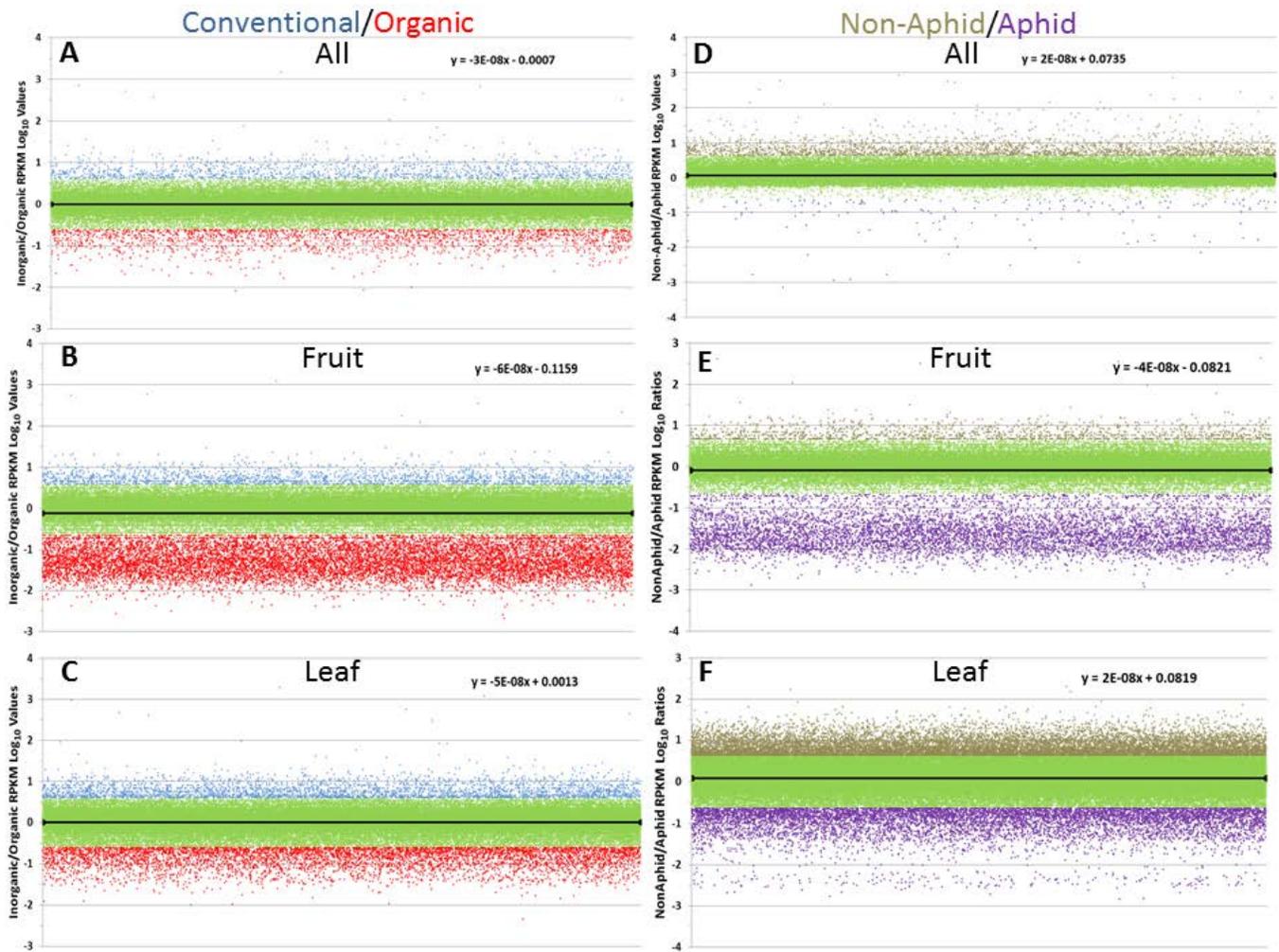


Figure 1. Complete transcriptome log₁₀ RPKM profiles. A. Fruit and Leaf combined transcriptome Conventional/Organic treated log₁₀ RPKM values profile. B. Conventional and organic treated Fruit transcriptome Conventional/Organic log₁₀ RPKM values profile. C. Conventional and organic treated Leaf transcriptome Conventional/Organic log₁₀ RPKM values profile. D. Fruit and Leaf combined transcriptome Non-aphid/aphid treated log₁₀ RPKM values profile. E. Non-aphid and aphid challenged Fruit transcriptome non-aphid/aphid log₁₀ RPKM values profile. F. Non-aphid and aphid challenged Leaf transcriptome non-aphid/aphid log₁₀ RPKM values profile. All figures have conventional over 5 fold expression in blue, organic over 5 fold expression in red, non-aphid over 5 fold expression in brown, aphid over 5 fold expression in purple and all non-differentially expressed values in green. Trend lines and slope formulas are shown to indicate treatment RPKM distributions.

Non-aphid and Aphid Challenged Fruit and Leaf Transcriptome Profiles under Conventional and Organic Treatments

The number of fruit conventional and fruit organic ESTs were similar with less than a 3% difference in the total numbers of ESTs in each treatment. Fruit in the organic treatment had 24% of the expressed ESTs greater than 5 fold difference compared to the conventional treatment where only 2% of the transcriptome was greater than 5 fold (Table 2 and Fig. 1B). Although the total organic numbers of

expressed ESTs were only 80% of the total expressed conventional ESTs, the number of ESTs 5 fold or higher in the organic treatment was 7.78% of the total organic EST numbers. The organic EST numbers 10 fold or greater totaled 2.76% of the total organic EST numbers. The conventional treatment ESTs that were 5 fold or higher were only 0.56% of the total conventional ESTs and 2.99% of the total conventional ESTs for 10 fold or higher ESTs (Table 2 and Fig. 1C). The number of ESTs in the conventional treatment for both the fruit and leaf samples were more numerous and diverse than the organic treatment but the relative number of organically treated ESTs in both the fruit and leaf had higher expression ratios in both the 5 fold and 10 fold or higher categories. These results indicate the conventional treatment induced a more varied transcriptional activity than the organic treatment and the organic treatment induced a less diverse genetic transcription profile but at higher levels of expression.

Table 2. Fruit only and leaf only transcriptome profile comparisons between conventional and organic treatments.

Fruit Conventional /Organic Treatment						
ESTs Higher in Conventional	49,518	Conventional ESTs > 10x	154	Conventional ESTs > 5x	1,031	
ESTs Higher in Organic	48,219	Organic ESTs > 10X	8,967	Organic ESTs > 5x	11,664	
Total Number of ESTs	109,807	Number Fruit ESTs	97,737	Non-differential ESTs	85,042	
Leaf Conventional /Organic Treatment						
ESTs Higher in Conventional	60,612	Conventional ESTs > 10x	339	Conventional ESTs > 5x	1,813	
ESTs Higher in Organic	48,648	Organic ESTs > 10X	1,345	Organic ESTs > 5x	3,785	
Total Number of ESTs	109,807	Number Leaf ESTs	109,260	Non-differential ESTs	103,662	

Fruit aphid challenged transcriptome EST numbers were 4% less than those of the fruit non-aphid challenged EST numbers (Table 3). As in the fruit organic treated transcriptome compared to the organically treated transcriptome, the ratio of ESTs greater than 5 fold (14.5% in the aphid treated and 2% non-aphid treated) and the ratio of ESTs 10 fold or higher (13% in the aphid treated and less than 1% non-aphid challenged transcriptome) the fruit aphid challenged transcriptome was less numerous and diverse than the fruit non-aphid challenged transcriptome but exhibited higher specific gene expression profiles (Figure 1E & Table 3). Due to similar transcriptome expression profiles, within 21% of each other, the more diverse control leaf transcriptome had slightly lower over-all percentages in expression amplitude. The control transcriptome 5 fold or higher profile was 13% of over-all ESTs and the aphid challenged transcriptome profile was 16% of the over-all aphid challenged transcriptome. 4% of the total non-aphid and 6.5% of the aphid challenged transcriptomes had 10 fold or higher EST RPKM expression values (Fig. 1F & Table 3).

Table 3. Fruit only and leaf only transcriptome profile comparisons between non-aphid and aphid challenge.

Fruit Non-Aphid/Aphid Samples						
ESTs Higher in Non-Aphid	51,387	Non-Aphid ESTs (>10x)	161	Non-Aphid ESTs (>5x)	943	

ESTs Higher in Aphid	49,459	Suspect Aphid ESTs (>10X)	6,494	Aphid ESTs (>5x)	7,182	
Total Number of ESTs	109,807	Number Leaf only ESTs	8,961	Non-differential ESTs	92,721	
Leaf Non-Aphid/Aphid Samples						
ESTs Higher in Non-Aphid	66,568	Non-Aphid ESTs >10x	2,418	Non-Aphid ESTs >5x	8,858	
ESTs Higher in Aphid	43,010	Aphid ESTs > 10X	2,817	Aphid ESTs >5x	6,723	
Total Number of ESTs	109,807	Number Leaf ESTs	109,578	Non-differential ESTs	93,997	

Comparison of the conventional and organic treatment fruit transcriptomes that were aphid challenged showed an 11% difference between the more diverse, fruit organic treatment aphid challenged and the fruit conventional treatment aphid challenged EST totals. There was quite a difference in the fruit conventional 5 fold or higher EST RPKM expression values, with 3% of the total reported ESTs, and the fruit organic of 22% of the total 5 fold or higher EST RPKM expression values. Fruit conventional treatment and aphid challenged did not increase in RPKM expression amplitude as the 10 fold or higher expression values only increased less than 1% at the 10 fold or greater level. In contrast, the 10 fold or higher RPKM expression profile of the fruit organic treatment aphid challenged transcriptome remained high with 18% of the transcriptome exhibiting a 10 fold or higher RPKM expression value (Fig. 2A & Table 4). The leaf organic treatment aphid challenged ESTs were 59% more numerous than the leaf conventional aphid challenged ESTs. 5 and 10 fold EST differential ESTs in the conventional leaf transcriptome were both lower than the organic leaf aphid challenged 5 and 10 fold differential ESTs by 11%, 4% conventional and 15% organic, and 7%, 2% conventional and 9% organic, for the 5 and 10 fold differential levels (Figure 2B & Table 4).

Table 4. Fruit only and leaf only transcriptome profile comparisons between conventional and organic treatments with aphid challenge.

Fruit Conventional/Organic Aphid Treatment						
ESTs Higher in Conventional	45,924	Conventional ESTs > 10x	211	Conventional ESTs > 5x	1,189	
ESTs Higher in Organic	51,425	Organic ESTs > 10X	9,208	Organic ESTs > 5x	11,294	
Total Number of ESTs	109,807	Number Fruit ESTs	97,349	Non-differential ESTs	84,866	
Leaf Conventional/Organic Aphid Treatment						
ESTs Higher in Conventional	39,165	Conventional ESTs > 10x	644	Conventional ESTs > 5x	1,711	
ESTs Higher in Organic	66,930	Organic ESTs > 10X	6,265	Organic ESTs > 5x	9,922	
Total Number of ESTs	109,807	Number Leaf ESTs	106,095	Non-differential ESTs	94,462	

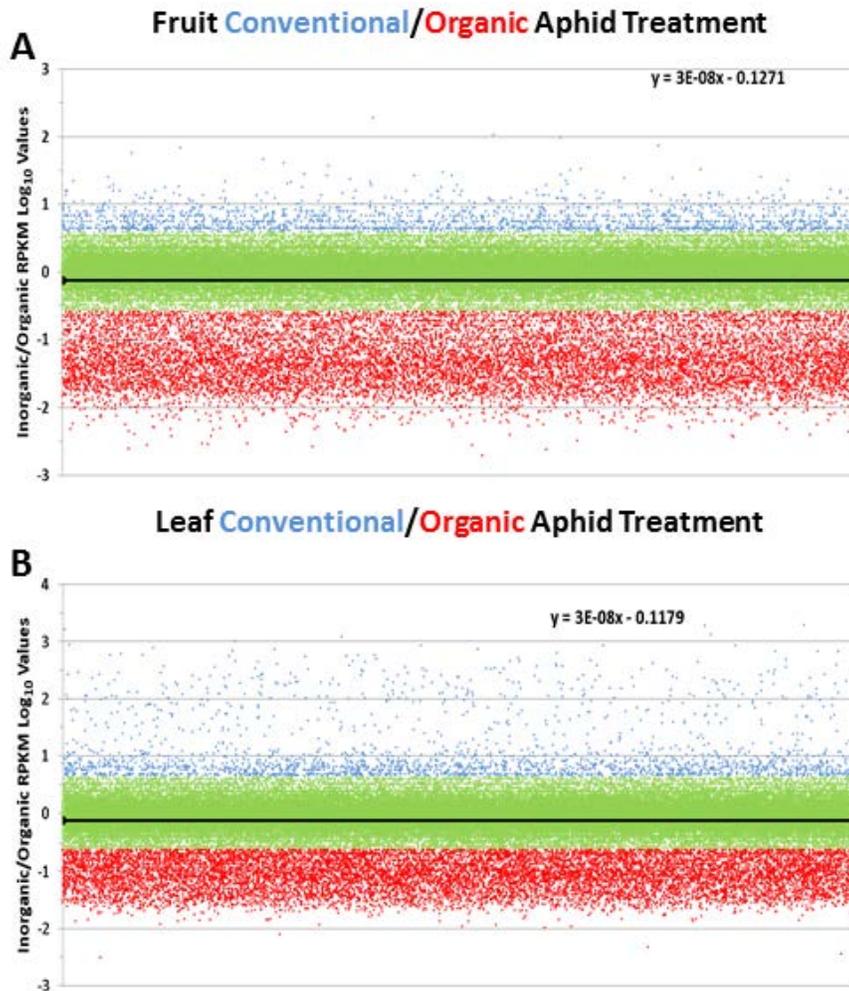


Figure 2. Complete transcriptome log₁₀ RPKM profiles. A. transcriptome Conventional/Organic treated aphid challenged log₁₀ RPKM values profile. **B.** Conventional and organic treated Fruit transcriptome Conventional/Organic log₁₀ RPKM values profile. All figures have conventional over 5 fold expression in blue, organic over 5 fold expression in red and all non-differentially expressed values in green. Trend lines and slope formulas are shown to indicate treatment RPKM distributions.

Transcriptome comparisons

The combined fruit and leaf transcriptome was most affected in total numbers by the aphid challenged treatment as compared to the conventional or organic treatments. The over-all transcriptome changes in the combined fruit and leaf transcriptomes had higher RPKM expression values over 5 fold in the organic compared to the conventional treatment and the aphid challenged over-all transcriptome over 5 fold RPKM expression levels were virtually non-existent in comparison to the modest percentage over 5 fold in the non-aphid transcriptome. The total fruit EST ratios were relatively the same between the conventional/organic comparisons and non-aphid/aphid challenged transcriptomes. Although the numbers of ESTs over 5 fold and 10 fold in the fruit only transcriptomes were not as diverse in the non-aphid/aphid challenged as compared to the conventional/organic treatments, both treatments in the fruit transcriptomes in regards to the over 5 fold and over 10 fold trends were the same with the organic and aphid challenged transcriptome RPKM values higher compared to their respective comparative treatments. The leaf transcriptomes total EST profiles were more varied compared to the fruit

transcriptomes with the leaf conventional ESTs more diverse than the organic treatment as was the non-aphid compared to the aphid challenged total EST numbers. The over 5 and over 10 fold numbers of ESTs in the conventional treatments remained similar between the fruit only and leaf only transcriptomes with the organic treatment having a greater effect on the fruit transcriptome in comparison to the leaf transcriptome. The diversity of the leaf only non-aphid transcriptome was larger in comparison to the leaf only aphid challenged transcriptome but the over 5 and over 10 fold EST numbers were much more similar between the non-aphid/aphid challenged treatments compared to the other transcriptome comparisons which indicated that while the aphid challenged leaf transcriptome was less diverse than the non-aphid leaf transcriptome the aphid challenged treatment had little effect on the amplitude of the ESTs.

The information presented here only reflects the quantity and amplitude of the genetic changes associated with each transcriptome compared to specific treatments and does not reflect which specific genes are modulated. Further work on homologous annotation of the ESTs by alignment and gene ontology would elucidate the specific genes and metabolic pathways correlated with the previously reported metabolite results between these different treatments.

- Publications, Handouts, Other Text & Web Products:
None.
- Outreach & Education Activities:
 1. R. Sharpe was an invited speaker in December of 2012 at Leibniz Universität Hannover Germany, for the Center for Applied Plant Science Seminar Series, where he presented “Utilizing NextGen Sequencing to Sequence Your Favorite Plant”.
 2. R. Sharpe was a speaker in the 2013 Molecular Plant Sciences Seminar Series, Washington State University. “Enabling Molecular Plant Sciences Through the Power of Next Generation Sequencing”.
 3. R. Sharpe was a speaker at the 2013 WSU Plant Sciences Retreat, Washington State University, where he presented “Building Genomic and Genetic Resources for Non Model Plant Species”.
 4. A. Dhingra, R. Sharpe and P. Andrews 2014 Transcriptomics approach to understand phytonutrient composition differences in plants grown under different nitrogen regimes. Int'l Phytomedomics and Nutriomics Consortium (ICPN) workshop. Plant and Animal Genome Jan 10-15, 2014 San Diego.

IMPACTS

- **SHORT-TERM:** Currently no definitive molecular evidence exists to support that organic soil treatment results in production of compounds that can impact human health. Data presented here is the first semblance of such evidence. We plan to disseminate this information via a manuscript. The project will increase our knowledge of the relationship between observed phytonutrient changes and underlying gene expression changes in plants when grown under organic soil fertility management and plant herbivory in a model crop plant (tomato). Such an interdisciplinary and comprehensive analysis remains to be done in the field of crop production. Once the analysis is completed, results of this study will be used to apply for grant funding in USDA's AFRI program in order to conduct a more comprehensive study, which includes field research.
- **INTERMEDIATE-TERM:** The knowledge generated from this BIOAg and subsequent federal projects will assist organic and sustainable producers to develop management plans for soil fertility and pest control that help them optimize the nutritional quality and yields of their products. The understanding of gene function and of markers associated with those genes will help plant breeders

in the breeding and selection of more efficient crop cultivars that optimize both nutritional quality and yields of crops grown using more sustainable farming practices.

- LONG-TERM: Increased consumption of foods with better nutritional quality should contribute to the health of American children and adults and reduce health care costs. Using more sustainable farming practices, especially of soil fertility and pest management, will enhance the quality of U.S. agroecosystems. Besides improving agricultural sustainability and public health, this project could also contribute to the long-term economic viability of American farmers.

ADDITIONAL FUNDING APPLIED FOR / SECURED

Inability to perform timely and comprehensive data analysis due to reduced levels of funding delayed completion of the work and a resulting manuscript which would have formed the basis of additional funding. We are attempting to complete the analysis as resources permit so that a USDA OREI can be submitted in 2015.

GRADUATE STUDENTS FUNDED

No graduate students were directly supported. Rick Sharpe, a graduate student supported by a NSF Grant to the PI performed much of the analysis.

RECOMMENDATIONS FOR FUTURE RESEARCH

The data analysis performed as part of this funding curtailed project supports the hypothesis that organic soil management results in differential genomic response which may result in production of phytonutrient dense crops. A higher resolution analysis of the data to understand gene expression networks and differentially expressed metabolic pathways is warranted prior to conducting further research. Ideally, additional large scale experiments under the two regimes should be performed to identify genotypes that perform superiorly under organic regimes to support the mission of the BioAg program.