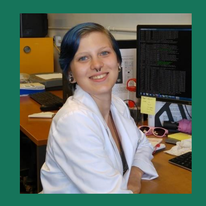
Identification of Differentially Expressed Genes for Mummy Berry (*Monilinia vaccinii-corymbosi*) Resistance in Blueberry

Ashley Yow[1]; Kathleen Burchhardt[2]; Marc Cubeta[2]; Hamid Ashrafi[1]
[1]NCSU Dept. of Horticultural Science, [2]NCSU Dept. of Plant Pathology



Introduction

- In 2015, combined fresh and frozen blueberry (*Vaccinium corymbosum*) sales were at the number 2 position for berry sales in the US, with a market value of over $1.6 billion.
- Mummy berry disease, caused by the fungal pathogen *Monilinia vaccinii-corymbosi*, is one of the most economically important diseases of blueberries. If left untreated, crop damage as high as 50% can occur during normal growing seasons.
- Next-Generation Sequencing (NGS) is a fast and effective tool for gene expression studies.
- RNA-Seq was used in this study for identifying genes potentially involved in the mummy berry disease infection process.
- This study will help to fill some of the molecular informational gap for this disease.

Objectives

- Use NGS technology for gene expression analysis in response to mummy berry disease infection.
- Do a comparative analysis between infected and noninfected tissues of the same cultivar.
- **Identify candidate genes for resistance to mummy berry disease in blueberry (*Vaccinium* sp.).**

Assembly Stats: *Monilinia vaccinii-corymbosi*		**Assembly Stats: Blueberry cv. Arlen**	
Assembled from 454 genome sequence data using automatic word and bubble size		Assembled from Illumina transcriptome sequence data using bubble size 150 and word size 31	
Statistic	**Length**	**Statistic**	**Length**
Minimum	200 bp	Minimum	300 bp
Maximum	14120 bp	Maximum	15726 bp
Average	754 bp	Average	865 bp
N50	838 bp	N50	1077 bp
Contig Count	18988	Contig Count	48349

Table 1. Assembly Statistics for transcriptome assembly of cv. Arlen and genome assembly of *M. vaccinii-corymbosi* fungal pathogen

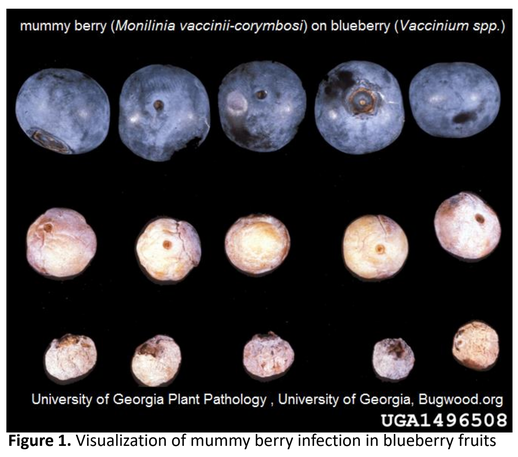


Figure 1. Visualization of mummy berry infection in blueberry fruits

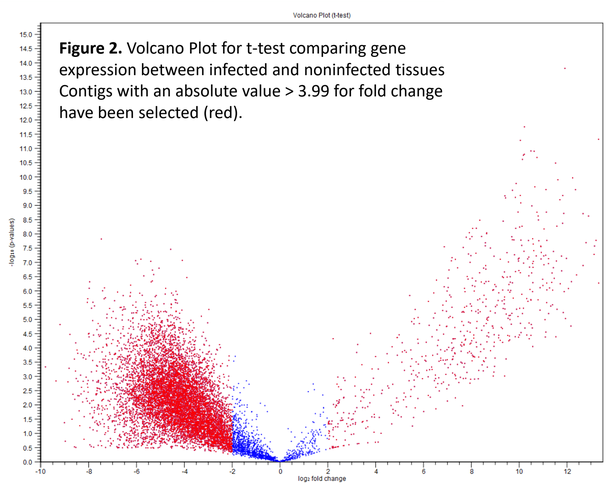


Figure 2. Volcano Plot for t-test comparing gene expression between infected and noninfected tissues Contigs with an absolute value > 3.99 for fold change have been selected (red).

Methods and Materials

- Samples were frozen in liquid N in the field and transferred to -80ºC freezer on dry ice.
- RNA was extracted from 16 tissues (12 unique) from susceptible cv. 'Arlen' using the Sigma-Aldrich Spectrum Plant Total RNA Kit.
- cDNA libraries were created using the KAPA mRNA Stranded Seq kit with a target insert size of 200-300 bp.
- BiooScientific NEXTflex DNA barcodes were used for multiplexing samples.
- Libraries were run at the NCSU Genomic Sciences Lab using the Illumina MiSeq 150bp SR (v3) service.
- CLC Genomics WB (v9.5) was used for QC, RNA-Seq Analysis, and transcriptome assembly.
- Roche 454 genomic reads were obtained for *Monilinia vaccinii-corymbosi* from Dr. Marc Cubeta and Kathleen Burchhardt and a genomic assembly was made using CLC Genomics WB.
- BLASTx 2.4.0+ was performed for the Arlen transcriptome assembly contigs using a local server.
- KEGG Pathway Mapping was performed in Blast2GO v 4.0.7 on the entire transcriptome assembly for Arlen.
- Genes of interest were filtered from the table for the t-test performed in CLC using a threshold p-value of ≤ 0.05 and a threshold fold change with absolute value > 3.99.

Results

- Out of an assembly of 48349 contigs total for cv. Arlen, 413 genes were selected for further analysis.
- These 413 genes fit within the parameters for p-value and fold-change for the t-test and their hit descriptions or GO names lists contained key words that were chosen based on previous studies.
  - Key words include: Jasmonic acid, Salicylic acid, Ethylene, Pathogenesis, Biotic stress, Defense response, Resistance, etc.
- Several fungal genes were found in the cv. Arlen transcriptome assembly, indicating that we still have fungal read contamination of our blueberry sequence data.

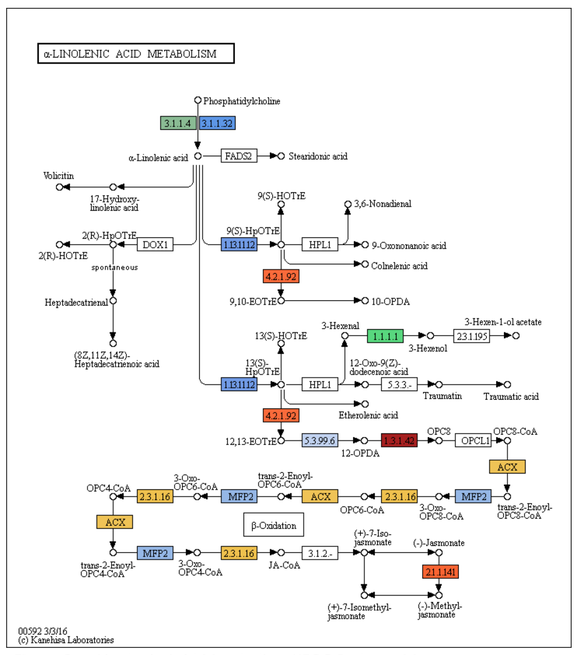


Figure 3. α-Linolenic Acid Metabolism KEGG Pathway Map
α-Linolenic acid biosynthesis is important for the first steps of synthesizing jasmonic acid. Jasmonic acid is a known signaling molecule for mediating responses to disease infection.

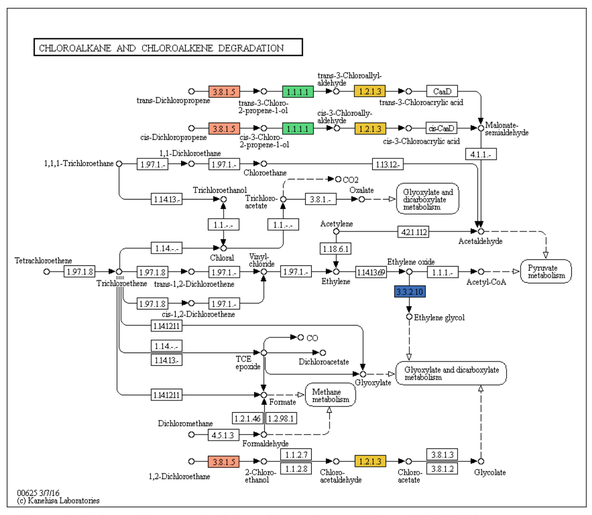


Figure 4. Chloroalkane and Chloroalkene Degradation KEGG Pathway Map
Contains other metabolic pathways important in defense response. Ethylene is also synthesized in this pathway, which is an important signaling hormone in mediating responses to stress.

Arlen Contig	t-test: Fold change	t-test: P-value	Description	e-value	Sim Mean
22	-13.3914	0.02913	jasmonic acid-amido synthetase JAR1-like [Nicotiana tabacum]	0	88.00%
6382	-143.829	0.00026	ethylene response sensor 1 [Vitis vinifera]	0	89.60%
1990	-80.7437	0.00013	ethylene-responsive small GTP-binding [Solanum lycopersicum]	1.1E-121	97.10%
10296	-31.0957	0.00036	Disease resistance [Theobroma cacao]	6.6E-113	91.70%
41024	1000000	1.2E-06	CMGC MAPK P38 kinase	1.4E-105	98.15%
11849	-21.3993	0.00449	pathogenesis-related 5-like	2.84E-86	75.30%
8474	4.710895	0.00964	Pathogenesis-related [Theobroma cacao]	4.1E-78	79.65%
22085	-5.14772	0.04867	disease resistance RPM1-like [Vitis vinifera]	5.27E-71	71.55%
44536	1000000	0.03932	salicylate hydroxylase	1.96E-66	86.50%
1425	-1000000	0.0215	resistance partial	4.5E-41	77.15%

Table 2. List of 10 candidate genes that are up or down-regulated during mummy berry disease infection
These genes are involved in jasmonic acid synthesis, salicylic acid synthesis, response to ethylene, MAPK signaling cascade, pathogenesis, and disease resistance.

Conclusions

- Based on the hit descriptions of our 413 selected genes, this data may likely contain promising results for candidate genes for resistance to mummy berry disease.
- The 413 genes found thus far in this study will need to be further filtered in order to further narrow down our list of potential candidate genes for resistance.
- Future work will focus on the addition of more replications and other cultivars along the resistance/susceptibility spectrum for comparison.
- More genomic data is also needed for the *M. vaccinii-corymbosi* pathogen, due to the continued contamination of our blueberry transcriptome data with fungal sequences. This will allow us to filter fungal sequences out of our blueberry data.

Contact

Ashley Yow
NC State University
Email: agyow@ncsu.edu
Website: https://blueberry.cals.ncsu.edu/current-lab-members/
Phone: (919) 637-9671

References

1. Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M., & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics, 21*(18), 3674-3676. doi:10.1093/bioinformatics/bti610
2. Dong, X. (1998). SA, JA, ethylene, and disease resistance in plants. *Current Opinion in Plant Biology, 1*(4), 316-323. doi:doi:10.1016/1369-5266(88)80053-0
3. Kunkel, B. N., & Brooks, D. M. (2002). Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology, 5*(4), 325-331. doi:10.1016/s1369-5266(02)00275-3
4. Lathrop, J. (2016, November). UMass Amherst research grant tackles mummyberry. *Fruit Growers News*, *55*(11), 52.
5. Meng, X., & Zhang, S. (2013). MAPK Cascades in Plant Disease Resistance Signaling. *Annual Review of Phytopathology, 51*(1), 245-266. doi:10.1146/annurev-phyto-082712-102314
6. Pullano, G. (2016, November). Blueberry consumption and sales keep pace with supply. *Fruit Growers News*, *55*(11), 46-47.
7. Rodrigues, C. M., Souza, A. A., Takita, M. A., Kishi, L. T., & Machado, M. A. (2013). RNA-Seq analysis of Citrus reticulata in the early stages of Xylella fastidiosa infection reveals auxin-related genes as a defense response. *BMC Genomics, 14*(1), 676. doi:10.1186/1471-2164-14-676
8. Rowland, L. J., Alkharouf, N., Darwish, O., Ogden, E. L., Polashock, J. J., Bassil, N. V., & Main, D. (2012). Generation and analysis of blueberry transcriptome sequences from leaves, developing fruit, and flower buds from cold acclimation through deacclimation. *BMC Plant Biology*, *12*, 46. doi:10.1186/1471-2229-12-46
9. Turner, J. G., Ellis, C., & Devoto, A. (2002). The Jasmonate Signal Pathway. *The Plant Cell, 14*, S153-S164. doi:DOI 10.1105/tpc.000679
10. Yang, F., Zhang, Y., Huang, Q., Yin, G., Pennerman, K. K., Yu, J., ... Guo, A. (2015). Analysis of key genes of jasmonic acid mediated signal pathway for defense against insect damages by comparative transcriptome sequencing. *Scientific Reports*, *5*, 16500. http://doi.org/10.1038/srep16500